



## Synthesis of oligo (15)--L- arabinofuranosides related to the plant polysaccharide pectin

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Synthesis of oligo (1→5)- $\alpha$ -L-  
arabinofuranosides related to the  
plant polysaccharide pectin

Mathilde Daugaard



## Preface

This thesis describes the research carried out from May 2011 to March 2016 as part of the Danish program to obtain a Ph.D. degree. The work was carried out at the Department of Chemistry at the Technical University of Denmark supervised by Professor Mads H. Clausen. The project has been part of the GlycAct project in collaboration with Professor William G.T. Willats at the University of Copenhagen. An external research stay was conducted in Professor Willats' group from June 2013 to October 2013 as part of the Ph.D.

Chapter one is a brief introduction to pectic polysaccharides and previously reported arabinofuranoside syntheses. Chapter two presents the synthetic strategy and challenges, which includes characterization of synthesized compounds. Chapter three describes the synthesis of two octasaccharides. In chapter four work done in the Professor Willats' group is outlined. This involves a linker system for oligosaccharides and a transglycosylation assay. Chapter five is a conclusion, chapter six contains experimental protocols and compound data, and chapter seven holds the references.

During the research stay at the University of Copenhagen some modeling of the interaction between chitosan oligosaccharides (COS) and oligogalacturonates (OGA) was performed and published, but not further described in this thesis.

Jozef Mravec, Stjepan K. Kračun, Maja G. Rydahl, Børge Westereng, Fabien Miart, Mads H. Clausen, Jonatan U. Fangel, **Mathilde Daugaard**, Pierre Van Cutsem, Henrik H. De Fine Licht, Herman Höfte, Frederikke G. Malinovsky, David S. Domozych and William G. T. Willats. "Tracking developmentally regulated post-synthetic processing of homogalacturonan and chitin using reciprocal oligosaccharide probes." *Development*, **2014**, 141, 1-10.

An article describing the work in chapter three is currently under preparation and included in appendix C:

**Mathilde Daugaard** and Mads H. Clausen. "Chemical synthesis of (1→5)- $\alpha$ -L-oligoarabinofuranosides." *In preparation*.

The review describing the chemical synthesis of oligosaccharides related to the plant cell wall is under preparation:

**Mathilde Daugaard**, Martin J. Pedersen, Mathias C. F. Andersen, Alexandra Zakharova and Mads. H. Clausen. Chemical Synthesis of Oligosaccharides related to the Plant Cell Wall. *Review proposal accepted by Chemical Reviews*.

## Acknowledgements

I would like to thank Professor Mads H. Clausen for hosting me as a Ph.D. student in his laboratory. Mads is also acknowledged for his expertise on chemistry, constructive feedback, and continuous support during my Ph.D. studies.

Professor William G.T. Willats from the University of Copenhagen is thanked for hosting me as a visiting graduate student. I felt very welcome in his group. I feel fortunate that I have obtained knowledge about different methods and procedures, than what I learn in the organic chemistry laboratory at DTU.

I would like to thank Charlotte Held Gotfredsen, Anne Hector, Tina Gustafson, Brian Brylle Dideriksen, Brian Ekman-Gregersen, and Philip Charlie Johansen for practical help acquiring NMR spectra, obtaining HRMS data, supporting the IR spectrometer, the melting point apparatus and the optical rotation apparatus and for ordering and delivering chemicals.

Past and present members of the Mads Clausen group are gratefully acknowledged for the positive working environment in the group, fruitful scientific discussions and unforgettable group dinners. I am especially thankful to Martin J. Pedersen for sharing many work hours in the laboratory while discussing chemistry and all other aspects of life. You have become a really good friend of mine. Thank you for proof reading parts of my thesis. Louise Kjærulff became a friend of mine, when we started at DTU over ten years ago. Thank you for a long friendship and for your many comments, when you proof read my thesis. I am also thankful for the corrections I got from Shahid I. Awan and Christine Kinnaert.

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Lastly and most importantly, I want to thank my family. All the support Karsten and I have received from my parents, his parents, and my sister has been invaluable. Thank you very much. My two children, Laura and Josefine, are thanked for helping me to get my thoughts off chemistry periodically. My greatest thanks of all go to my loving and forgiving husband Karsten. I love you. Thank you for supporting me and my project and for being a great father to our kids.

## Abstract

A strong fundamental understanding of plant biology is essential for meeting society's growing demand for safe and nutritious food, dietary fibers, clothes, and renewable energy sources for an increasing global population. The plant cell wall is one of the main targets for biotechnological research, as it represents almost 50% of plant biomass.

A major constituent of the plant cell wall is different complex polysaccharides. The knowledge about their detailed structure and function on a molecular level is far from complete, and structural studies are complicated by the great complexity of the cell wall. The diversity of polysaccharides and the microheterogeneity in the cell wall make it extremely challenging to isolate well-defined compounds after partial degradation of plant material. Chemical synthesis, on the other hand, is capable of producing structurally diverse oligosaccharides of excellent purity, and in larger quantities.

The objective of the present study is to design and execute chemical syntheses of well-defined pectic oligosaccharides. These can serve as models for the more complex polysaccharide network found in the plant cell wall. The chemical synthesis of two branched structures of oligo (1→5)- $\alpha$ -L-arabinofuranosides that are prominent side chains in RG-I is presented. By employing a disaccharide donor, the number of glycosylation reactions was reduced significantly and late stage regioselective deprotection made it possible to introduce different sidechains in the oligosaccharides.

The work done during an external research stay at University of Copenhagen is also described. This includes the implementation of a covalent linker system as an alternative to bovine serum albumin (BSA) for oligosaccharides, as well as the development of a microarray-based transglycosylation assay capable of screening for novel glycosyl transferase/hydrolase activities.

## Dansk Resumé

For at imødekomme en global voksende befolknings efterspørgsel på f.eks. fødevarer, kostfibre, beklædning og vedvarende energikilder, er en fundamental forståelse af plantebiologi essentiel. I den forbindelse er plantecellevæggen et interessant forskningsområde, da den repræsenterer halvdelen af planter biomasse.

Hovedbestanddelen af plantecellevæggen er forskellige komplekse polysakkarider, men det nuværende kendskab til dennes detaljerede struktur og funktion på et molekylært niveau er langt fra fuldkommen. Mikroheterogeniteten og mangfoldigheden af polysakkarider i cellevæggen gør det utrolig kompliceret at isolere veldefinerede stoffer efter delvis nedbrydning af plantemateriale. Ved hjælp af kemisk syntese er det derimod muligt at producere strukturelt forskellige oligosakkarider i fremragende renhed og i større mængder.

Formålet med dette studie har været at designe og udføre kemiske synteser af strukturelt veldefinerede oligosakkarider kendt fra pektin, som sidenhen kan tjene som modeller for de komplekse polysakkarider i plantecellevæggen. Den kemiske syntese af to forgrenede oligo (1→5)- $\alpha$ -L-arabinofuranosider, der er sidekæder i RG-I, er præsenteret. Det var muligt at holde antallet af glykosyleringsreaktioner nede ved at benytte en disakkarid donor. En regioselektiv afbeskyttelse på et sent tidspunkt i syntesen, gjorde det muligt at introducere forskellige sidekæder på to lineære oligosakkarider.

Arbejdet udført i forbindelse med et eksternt forskningsophold på Københavns Universitet er også beskrevet. Dette inkluderer implementeringen af et linker system for oligosakkarider som alternativ til bovint serum albumin (BSA) og udviklingen af et mikroarraybaseret transglykosyleringsassay i stand til at screene for ny glykosyltransferase/hydrolase aktivitet.

## List of Abbreviations

AB spin	AB spin systems
Ac	Acetyl
AceA	Aceric acid
Api	Apiose
Aq.	Aqueous
Ara	Arabinose
Bn	Benzyl
br	Broad singlet
BSA	Bovine serum albumin
Bu	Butyl
Bz	Benzoyl
ClAc	Chloroacetyl
CMPI	2-Chloro-1-methylpyridinium iodide
COS	Chitosan oligosaccharide
CSA	Camphor-10-sulfonic acid
d	Doublet
dd	Doublet of doublets
ddd	Doublet of doublet of doublets
DABCO	1,4-Diazabicyclo[2.2.2]octane
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
Dha	2-Keto-3-deoxy-D-lyxo-heptulosaric acid
DMAP	4-(Dimethylamino)pyridine
DMF	<i>N,N</i> -Dimethylformamide
DMSO	Dimethyl sulfoxide
DQF-COSY	Double quantum filtered correlation spectroscopy
dt	Doublet of triplets
Et	Ethyl
ESI	Electron spray ionization
Equiv.	Equivalents
GH	Glycosyl hydrolase
h	Hours
HG	Homogalacturonan
HMBC	Heteronuclear multiple bond correlation spectroscopy



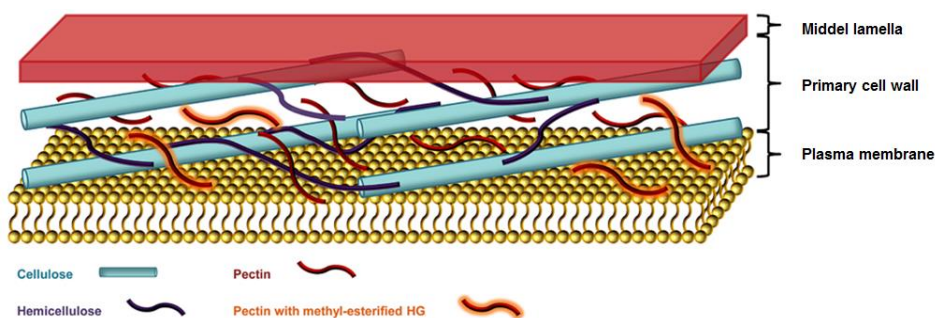
HPLC	High-performance liquid chromatography
HRMS	High-resolution mass spectrometry
HSQC	Heteronuclear single quantum coherence
IR	Infrared
Kdo	2-Keto-3-deoxy-D-manno-octulosonic acid
LAM	Lipoarabinomannan
m	Multiplet
mAb	Monoclonal antibodies
MALDI	Matrix-assisted laser desorption/ionization
Man	Mannose
Me	Methyl
mp	Melting points
MS	Mass spectrometry
NAP	2-Naphthylmethyl
NBS	<i>N</i> -Bromosuccinimide
NIS	<i>N</i> -Iodosuccinimide
NHS-biotin	<i>N</i> -Hydroxysuccinimide ester
NMR	Nuclear magnetic resonance
OGA	Oligogalacturonate
oligo	Oligosaccharide
p	Pentet/page
P	Protecting group
Ph	Phenyl
PMT	Photo multiplier tube
po	Polysaccharide
ppm	Parts per million
Pr	Propyl
q	Quartet
RG	Rhamnogalacturonan
s	Singlet
sat.	Saturated
<i>t</i>	<i>Tert</i>
t	Triplet
TBAF	tetra- <i>n</i> -Butylammonium fluoride
TBS	<i>tert</i> -Butyldimethylsilyl

TBDPS	<i>tert</i> -Butyldiphenylsilyl
td	Triplet of doublets
TES	Triethylsilyl
TFA	Trifluoroacetic acid
Tf	Trifluoromethanesulfonyl
THF	Tetrahydrofuran
TIPDS	Tetraisopropylidisiloxane-1,3-diyl
TIPS	Triisopropylsilyl ether
TLC	Thin layer chromatography
TMS	Trimethylsilyl
Tol	Tolyl, <i>p</i> -methylphenyl
Tr	Triphenylmethyl
UV	Ultraviolet
XET	Xyloglucan endotransglycosylase
XGA	Xylogalacturonan
XG	Xyloglucan
Xyl	Xylose

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# 1 Introduction

Pectins are an important group of polysaccharides found in the plant cell wall (Figure 1). Pectins contribute to cell functions such as support, defense, signaling, and cell adhesion and they play an important role as a food additive, serving as stabilizer and thickening agent. Pectin polysaccharides are composed of three different structural domains known as rhamnogalacturonan I (RG-I), rhamnogalacturonan II (RG-II), and homogalacturonan (HG).



**Figure 1** Representation of the plant cell wall printed with permission from Willats *et al.*<sup>1</sup>

In an effort to improve the understanding of the pectin, increasing attention has, in recent years, been given to the structure and function of plant wall polysaccharides.<sup>2-4</sup>

The structure of pectin has long been a focus of interest in the Mads H. Clausen group. Mads H. Clausen completed the synthesis of five partially methyl-esterified hexagalacturonates from the HG part of pectin.<sup>5</sup> Later Alexandra Zakharova, a Ph.D. student in the Mads H. Clausen group, synthesized a hexasaccharide fragment of the RG-I backbone,<sup>6</sup> and Mathias C. F. Andersen, a Ph.D. student supervised by Mads H. Clausen, synthesized a variety of D-galactosides, which are found as prominent side chains in the RG-I structure.<sup>7</sup>

One way to examine structurally complex molecules, such as pectin, is to study the smaller constituents individually, like done for the galacturonates and the galactosides.<sup>8-10</sup> To gain more information about the structure of pectin, another prominent side chain of RG-I, L-arabinofuranoside, was desired.

Chemical synthesis provides reliable routes to compounds with well-defined structures in multi-milligram scale. De novo synthesis of oligosaccharides offers the possibility of great structural diversity in the target molecules. Chemical synthesis of oligosaccharides is, however, a laborious process, since

no straightforward methods exist for oligosaccharide synthesis, as opposed to e.g. the solid phase synthesis of peptides.<sup>11</sup> One of the main limitations in oligosaccharide synthesis is distinction of the many hydroxyl groups on the monosaccharides, which require different protecting groups. The other main limitation involves the glycosylation reactions, which are often only modest yielding, and in which stereocontrol can be challenging. The goal is to prepare target molecules in minimum 95% purity, of which the impurities should mainly come from the protecting groups or the chemical reagents used, which most often do not affect the following biological tests of the oligosaccharides.

### 1.1 Pectin

Pectin was first discovered as “a peculiar substance” in tamarind fruit in 1790 by Vauquelin,<sup>12</sup> and later described by Braconnot as “pectin” in 1825.<sup>13</sup> Pectin arrives from the Greek word pektikos, which means congeal, solidify or curdle, which is the exact property of pectins: their ability to jellyfy. But pectic polysaccharides possess much more important biological roles than acting as jellyfying agent in the food industry. It is generally understood that the mechanical strength and physical properties of primary cell walls are largely dependent on the pectic polysaccharides.<sup>14–16</sup>

Pectins are also useful in the pharmaceutical and cosmetic industries for the manufacture of encapsulated drugs and skin-care products.<sup>17,18</sup>

Pectins constitute an important part of the plant cell wall.<sup>19</sup> Investigating the structure of pectin can contribute with more knowledge of properties such as cell wall biosynthesis and protein-carbohydrate interactions. Pectins are built up of different regions: RG-I, homogalacturonan (HG), xylogalacturonan (XGA), and rhamnogalacturonan II (RG-II) (Figure 2).

Figure 2 illustrates a general structure of pectin in a cartoon fashion for overview purposes. Each monosaccharide holds a different color; D-enantiomers are fully colored whereas L-enantiomers are shaded. Whether the monosaccharide occurs in the furanose or the pyranose form is illustrated by the geometry of each monosaccharide, where each corner represents either a carbon or an oxygen atom. This gives a simple overview of which position links one monosaccharide to the next one. The glycosidic linkages between two monosaccharides are illustrated with a dot for  $\alpha$ -linkages and no dot for  $\beta$ -linkages. This cartoon fashion will be used throughout the thesis for overview purposes when relevant.

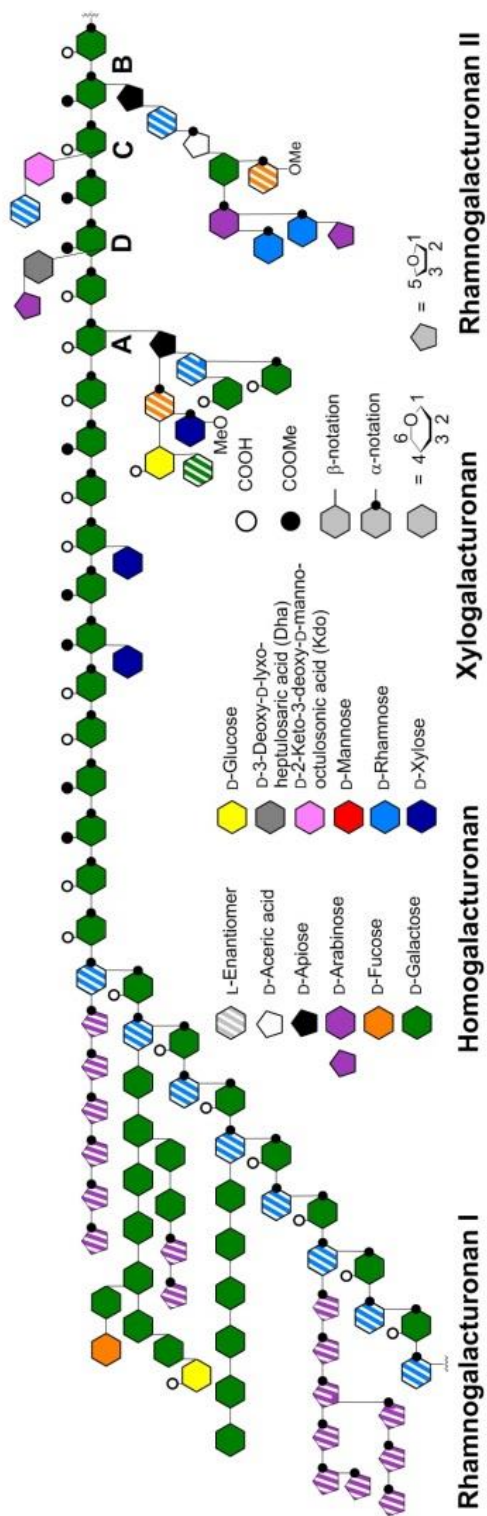


Figure 2 Representation of pectin with its four parts: RG-I, HG, XGA, and RG-II.

I am aware of the recently published article in Chemical and Engineering News, which presents a different glycan notation as new standard.<sup>20</sup> We, however, feel that the representation in Figure 2 provides an easier overview using less space.

HG is the most abundant pectic polysaccharide. It consists of a linear chain of (1→4) linked  $\alpha$ -D-galactopyranuronic acid residues, with a length of around 100-200 galacturonic acid units of which some of the carboxylic acids are methyl esterified. The degree of esterification depends of the specific plant and the position of the cell in the plant tissue.<sup>21</sup> The methylation degree is well known to influence the gelling character as well as other properties. HG is referred to as the smooth region of pectin.

RG-II is a minor component in pectin. It has a backbone of minimum eight and up to 15 (1→4) linked  $\alpha$ -D-galactopyranuronic acid units with four distinct side chains (A-D, shown on Figure 2). The side chains are constructed from 13 different monosaccharides, of which many are rare sugars, such as L-aceric acid (AceA; 3-C-carboxy-5-deoxy-L-xylose), 2-keto-3-deoxy-D-manno-octulosonic acid (Kdo), 2-keto-3-deoxy-D-lyxo-heptulosaric acid (Dha), D-apiose (Api; 3-C-(hydroxymethyl)- $\beta$ -D-erythrose), L-galactose, 2-O-methyl-D-xylose and 2-O-methyl-L-fucose. The monosaccharides are connected by over 20 different linkages.<sup>22</sup> Borate forms ester bonds with the apiosyl residues of side chain A of two RG-II monomers to generate crosslinked RG-II dimers.<sup>23</sup> Studies have shown that RG-II is conserved across the plant kingdom and increasing evidence supports that RG-II and its borate crosslinks are critical for plant growth and development by supporting e.g. cell adhesion and mechanical strength.<sup>14</sup>

XGA is also a minor constituent of pectin. It consists of a (1→4) linked  $\alpha$ -D-galactopyranuronic acid, which is substituted with  $\beta$ -D-xylose at the O-3 position.<sup>24</sup> The 3-linked xylose has occasionally been found to be further substituted at O-4 with an additional  $\beta$ -linked xylose. Some studies suggest that XGA may help HG be more resistant to degradation by endopolygalacturonases produced during pathogen attack.<sup>25</sup>

### 1.1.1 Rhamnogalacturonan I

RG-I is the most abundant part of pectin after HG. RG-I holds a backbone of alternating galacturonic acid and rhamnose monosaccharides branched on the rhamnose residues with side chains of linear and branched galactans, arabinans, and arabinogalactans.<sup>21</sup> Along with the RG-II region these are called the hairy regions of pectin as opposed to the smooth unbranched HG region .

The degree of branching and the length and composition of the side chains depend on the plant source. The galactan side chains are mostly linear (1→4) linked  $\beta$ -D-galactosides, whereas the arabinan side chains are (1→5) linked  $\alpha$ -L-arabinofuranoside chains with possibility of L-arabinan sidechains in the 2- and/or 3-positions.<sup>26,27</sup> The arabinogalactan side chains either contain a backbone of (1→4) linked  $\beta$ -D-galactopyranoside substituted mainly in the 3-position with L-arabinofuranosides.<sup>28</sup>

## 1.2 Beneficial effects of L-arabinose

Initially our interest in L-arabinofuranosides arose from their presence in pectin and our urge to better understand the structure of this complex polysaccharide. Later we realized that literature holds several examples of beneficial health effect of L-arabinose.

Oligo (1→5)- $\alpha$ -L-arabinofuranosides derived from sugar beet pectin have been reported to possess a wide range of biological activity profiles including immunological activity<sup>29,30</sup> and being a promising dietary supplement for improvement of human intestinal health.<sup>31,32</sup>

Recently the monosaccharide L-arabinose has been found to pose selective intestinal sucrase inhibition effect<sup>33</sup> as well as protective effects in high-carbohydrate, high-fat diet-induced metabolic syndrome in rats,<sup>34</sup> and great usage in tumor therapy as inducer of bacterial gene expression in combination with a molecular switch.<sup>35,36</sup>

Gao *et al.* reported that arabinose can increase or decrease the interaction between galactans and galectin-3 depending on their location within the RG-I molecule.<sup>37</sup> Galectin-3 is a galactans-binding protein considered as a diagnostic marker and a target protein for cancer treatment.<sup>38</sup>

## 1.3 L-Arabinofuranoside structures found in Nature

L-Arabinofuranosides are found in the cell wall of many different plant sources and green alga. Table 2 offers an overview of many of the L-arabinofuranosides reported in Nature.

The arabinan side chains in pectin are generally thought to be directly attached to the rhamnogalacturonan backbone, as illustrated in many schematic representations of pectin/RG-I.<sup>39–43</sup> However, very limited evidence for this claim has been reported up until now. To the best of our knowledge the only clear evidence for an arabinose-rhamnose link in literature is found in the work by Wu *et al.*<sup>44</sup> They reported a cross peak between the anomeric proton H-1 of the arabinosyl residue and C-4 of the rhamnosyl residue in the heteronuclear



multiple bond correlation spectroscopy (HMBC) spectrum of an oligomer obtained from boat fruit.

L-Arabinofuranosides are mainly found to be (1→5) linked with occasional branchings on the 2- and/or the 3-position. Recently a (1→3)  $\alpha$ -linked chain was obtained and characterized,<sup>27</sup> and occasionally terminal  $\beta$ -arabinofuranoside residues have been detected in pectic substances.<sup>45</sup> The degree of polymerization of the arabinans varies with the stage of ripening of a fruit, as described for the olive fruit by Ferreira *et al.*, Vierhuis *et al.*, and Mafra *et al.*<sup>46–48</sup>

**Table 1** Arabinans reported in different plant sources. The table is an expanded version of a similar table reported by Ding *et al.*, who also presented an overview of linkage types reported in each case.<sup>49</sup>

Plant source	Reference
Mandarin ( <i>Citrus unshiu</i> )	Coll-Almela <i>et al.</i> 2015 <sup>50</sup>
Ma Huan ( <i>Ephedra sinica</i> )	Xia <i>et al.</i> 2015 <sup>27</sup>
Amaranth ( <i>Amaranthus hypochondriacus</i> )	Wefers <i>et al.</i> 2015 <sup>51</sup>
Potato ( <i>Solanum tuberosum</i> )	Khodaei and Karboune 2013 <sup>52</sup>
Styrian oil-pumpkin ( <i>Cucurbita pepo</i> )	Košťálová <i>et al.</i> 2013 <sup>53</sup>
Quinoa ( <i>Chenopodium quinoa</i> )	Cordeiro <i>et al.</i> 2012 <sup>54</sup>
Nata Karanja ( <i>Caesalpinia bonduc</i> ) seed	Mandal <i>et al.</i> 2011 <sup>55</sup>
Boat-fruit ( <i>Semen Sterculiae lychnophorae</i> )	Wu <i>et al.</i> 2007 <sup>56</sup> and Wu <i>et al.</i> 2013 <sup>44</sup>
Green alga ( <i>Chlamydomonas reinhardtii</i> )	Bollig <i>et al.</i> 2007 <sup>57</sup>
Roots of ( <i>Echinacea pallida</i> )	Thude and Classen 2005 <sup>58</sup>
Almond ( <i>prunus dulcis</i> ) seed	Dourado <i>et al.</i> 2004 <sup>29</sup> and Dourado <i>et al.</i> 2006 <sup>59</sup>
Honey locust ( <i>Gleditsia triacanthos</i> ) seed	Navarro <i>et al.</i> 2002 <sup>60</sup>
Leaves from Oleander ( <i>Nerium indicum</i> )	Dong and Fang 2001 <sup>61</sup>
Brazilian firetree ( <i>Schizolobium parahybae</i> ) seed	Petkowicz <i>et al.</i> 1998 <sup>62</sup>
Cassia ( <i>fastusa</i> ) seed	Petkowicz <i>et al.</i> 1998 <sup>62</sup>
<i>Schizolobium amaronicum</i> seeds	Petkowicz <i>et al.</i> 1998 <sup>62</sup>
Rapeseed ( <i>Brassica napus</i> )	Eriksson <i>et al.</i> 1996 <sup>63</sup>
Hemp bast fibres ( <i>Cannabis</i> )	Vignon and Garcia-Jaldon 1996 <sup>64</sup>
Chinese herbal medicine (Kampo medicine)	Yamada 1995 <sup>65</sup>
Olive ( <i>Olea europaea</i> )	Coimbra <i>et al.</i> 1994, <sup>66</sup> Huisman <i>et al.</i> 1996, <sup>67</sup> Vierhuis <i>et al.</i> 2000, <sup>47</sup> Mafra <i>et al.</i> 2001, <sup>68</sup>

- Cardoso *et al.* 2002,<sup>45</sup> Vierhuis *et al.* 2003,<sup>69</sup> Ferreira *et al.* 2006,<sup>46</sup> and Cardoso *et al.* 2007<sup>70</sup>
- Lupin (*Lupinus angustifolius*) cotyledon  
 Pea hull (*Pisum sativum*)  
 Red wine  
 Guapuruvu (*Schizolobium parahybum*)  
 Leaves from (*Ginkgo biloba*)  
 Pigion pea (*Cajanus cajan*) cotyledon  
 Flax (*Linum usitatissimum*)  
 Parsnip (*Pastinica sativa*)  
 Mung bean (*Vigna radiate*)  
 Cowpea (*Vigna sinensis*)  
 Pollen tubes from Tobacco (*Nicotiana glauca*)  
 Horsebean (*Vicia faba*) root  
 March mallow root (*Althaea officinalis*)  
 Grape (*Vitis vinifera*)  
 Prickly pear (*Opuntia ficus-indica*)  
 Cabbage (*Brassica oleracea* Var. *capitata*)  
 Inner bark of young stems (*Rosa glauca*)  
 Roots and leaves of (*Althaea officinalis*)  
 Sesame (*Sesamum indicum*)  
 Rape seed (*Brassica napus*)  
 White willow (*Salix alba*) bark  
 Rape seed (*Brassica campestris*)  
 Jujube fruit (*Ziziphus vulgaris*)  
 Soybean (*Glycine max*)  
 Lemon peel (*Citrus limon*)  
 Apple (*malus domestica*)
- Cheetham *et al.* 1993<sup>71</sup>  
 Ralet *et al.* 1993<sup>72</sup>  
 Belleville *et al.* 1993<sup>73</sup>  
 Zawadzki-Baggio *et al.* 1992<sup>74</sup>  
 Kraus 1991<sup>75</sup>  
 Swamy and Salimath 1991<sup>76</sup>  
 Davis *et al.* 1990<sup>77</sup> and Ding *et al.* 2015<sup>49</sup>  
 Siddiqui and Emery 1990<sup>78</sup>  
 Herve du Penhoat and Michon 1987<sup>79</sup>  
 Muralikrishna and Tharanathan 1986<sup>80</sup>  
 Rae *et al.* 1985<sup>81</sup>  
 Joseleau *et al.* 1983<sup>82</sup>  
 Capek *et al.* 1983<sup>83</sup>  
 Villettaz *et al.* 1981<sup>84</sup> and Saulnier and Brillouet 1988<sup>85</sup>  
 McGarvie and Parolis 1981,<sup>86</sup>  
 Vignon *et al.* 2004,<sup>87</sup> and Habibi *et al.* 2005<sup>88</sup>  
 Stevens and Selvendran 1980<sup>89</sup>  
 Joseleau *et al.* 1977<sup>90</sup>  
 Tomoda *et al.* 1977,<sup>91</sup> Tomoda *et al.* 1980,<sup>92</sup> and Tomoda *et al.* 1981<sup>93</sup>  
 Wankhede and Tharanathan 1976<sup>94</sup>  
 Norton and Harris 1975<sup>95</sup>  
 Karacsonyi *et al.* 1975<sup>96</sup>  
 Siddiqui and Wood 1974<sup>97</sup> and Larm *et al.* 1975<sup>98</sup>  
 Tomoda *et al.* 1973<sup>99</sup>  
 Aspinall and Cottrell 1971<sup>100</sup>  
 Aspinall and Cottrell 1970<sup>101</sup>  
 Barrett and Northcote 1965,<sup>102</sup>  
 Churms *et al.* 1983,<sup>103</sup> and Aspinall and Fanous 1984<sup>104</sup>

Mustard ( <i>Brassica juncea</i> ) seed meal	Hirst <i>et al.</i> 1965 <sup>105</sup> and Tharanathan <i>et al.</i> 1985 <sup>106</sup>
Maritime pine ( <i>Pinus pinaster</i> )	Roudier and Eberhard 1965 <sup>107</sup>
Guava ( <i>Psidium guajava</i> )	Sengupta <i>et al.</i> 1965 <sup>108</sup>
Sugar beet ( <i>Beta vulgaris</i> )	Hirst and Jones 1948, <sup>109</sup> Hough and Powell 1960, <sup>110</sup> Hullar 1965, <sup>111</sup> Guillon and Thibault 1989, <sup>112</sup> Guillon and Thibault 1989, <sup>113</sup> Sakamoto and Sakai 1995, <sup>42</sup> and Oosterveld <i>et al.</i> 2000 <sup>114</sup>
Peanut ( <i>Arachis hypogea</i> )	Hirst and Jones 1947 <sup>115</sup>
Psyllium Seed ( <i>Plantago psyllium</i> )	Anderson and Fireman 1935 <sup>116</sup>
Cress seed ( <i>Lepidium sativum</i> ) mucilage	Bailey 1935 <sup>117</sup>
Seed of White mustard ( <i>Brassica alba</i> )	Bailey and Norris 1932, <sup>118</sup> Rees and Richardson 1966, <sup>119</sup> and Rees and Steele 1966 <sup>120</sup>
Quince Seed ( <i>Cydonia vulgaris</i> )	Renfrew and Cretcher 1932 <sup>121</sup>

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## 1.4 Previously synthesized oligo arabinofuranosides

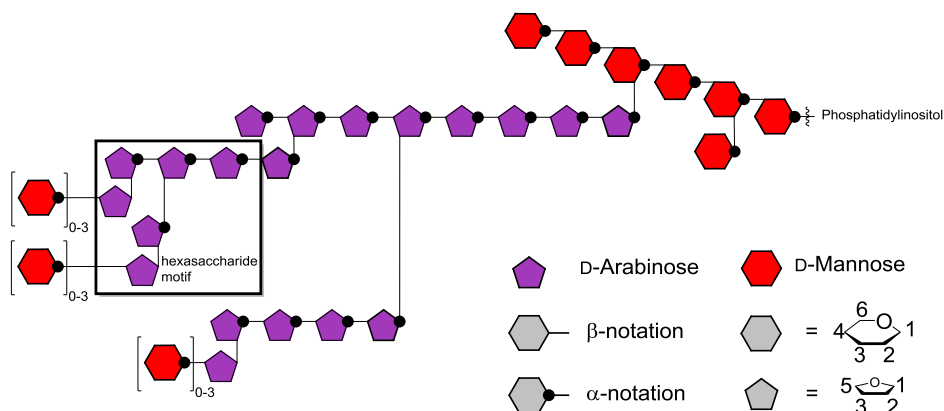
Chemical synthesis is, as previously described, an often essential method to acquire compounds with well-defined chemical structures, with target molecules originating in Nature. This section offers an overview of the reported approaches to obtain oligomers of D- and L-arabinofuranosides. These are enantiomers, which mean that they are the mirror images of each other. In many aspects enantiomers behave identical e.g. possessing identical  $R_f$  values, NMR spectra and melting points. When exposed to a chiral environment, two enantiomers, however, do not perform alike, therefore introducing the correct enantiomer in a biological context is crucial, and synthesizing L-arabinofuranosides to investigate the plant cell wall is essential. Since enantiomers perform alike in chemical reactions, inspiration to synthetic strategies can very conveniently and sensibly be gained from the enantiomer.

### 1.4.1 Chemical synthesis of oligo D-arabinofuranosides

Extensive work has been carried out synthesizing oligomers of D-arabinofuranosides that make up part of the cell wall of *Mycobacterium tuberculosis*. These bacteria causes the infectious disease tuberculosis, which is one of the leading causes to deaths worldwide.<sup>122</sup>

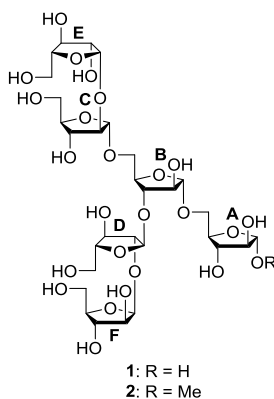
A major mycobacterial cell wall glycan is lipoarabinomannan (LAM), which is a lipidated polysaccharide that plays a critical role in mycobacteria-host interactions (Figure 3). The following is meant as a short introduction to the

synthetic strategies used in this work. Many more references can be found in the cited articles and in Cao and Williams' review about the chemical approaches for the study of the mycobacterial glycolipids phosphatidylinositol mannosides, lipomannan and lipoarabinomannan.<sup>123</sup>



**Figure 3** Schematic structure of the composite structure of lipoarabinomannan.<sup>124</sup>

A main arabinan structure in lipoarabinomannan is the hexasaccharide motif capturing the non-reducing end of the big oligosaccharide structure, marked on Figure 4. The terminal (1→2)- $\beta$ -arabinofuranose residue might be capped with mannopyranosyl oligosaccharides. Initially much focus was put on synthesizing the branched hexasaccharide motif **2** (Figure 4). With this chemically synthesized structure, it was found that one of the major antibodies generated against mycobacterial LAM binds this hexasaccharide.<sup>125</sup> Based on these findings an assay has been developed, which can be used to discriminate between tuberculosis and non-tuberculosis patients.<sup>126</sup>



**Figure 4** Hexaarabinoside motif **2** found at the non-reducing termini of lipoarabinomannan.

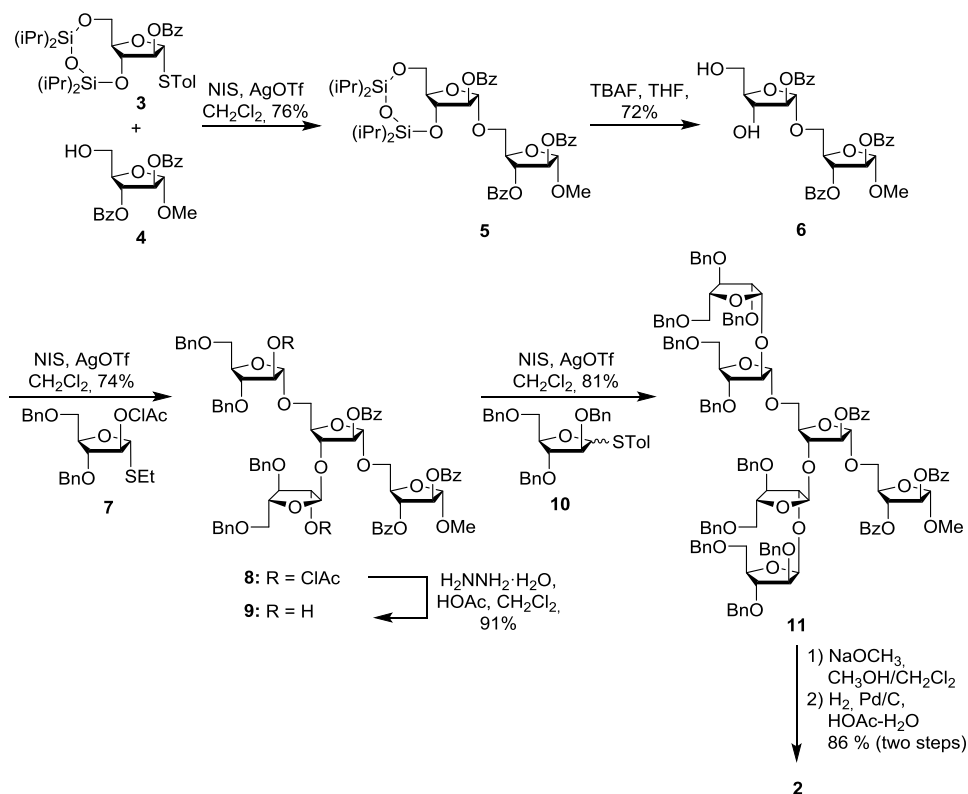
The synthetic strategy of the “middle”-tetrasaccharide, holding monosaccharides A, B, C, and D (Figure 4), which are connected by  $\alpha$ -linkages, was first established by Ayers *et al.* in 1998.<sup>127</sup> The reducing end of the molecule was capped with a methyl group. Thioglycosides were chosen as the glycosyl donors because of their hydrolytic stability. The reactions were performed at 0 °C promoted by NIS and silver triflate (AgOTf) and resulted in high yields and excellent stereoselectivity (Scheme 1). The stereoselectivity for the  $\alpha$ -linkage was assisted by neighboring group participations and the difficulties in forming a  $\beta$ -D-arabinofuranosyl-linkage as discussed below.

Due to the symmetric nature of the hexaarabinoside motif, monosaccharide **C** and **D** are often introduced simultaneously.<sup>128</sup> Although stepwise introduction of monosaccharide **C** and **D** also have been shown.<sup>129</sup>

Monosaccharide **E** and **F** are linked with  $\beta$ -linkages and these are notoriously difficult to synthesize, similar to the  $\beta$ -D-mannopyranosyl linkage which is a stereochemical analog. The *cis* orientation of the C-1 and C-2 groups in the two molecules prohibits the advantage of neighboring group participation using glycosyl donors with C-2 acyloxy groups because this produces 1,2-trans glycosides. Donors holding a nonparticipating group at C-2 also mainly result in 1,2-trans glycosides since both stereoelectronic and steric effects favor the formation of the  $\alpha$ -glycoside, in these cases. Recently various methods to overcome the challenge with  $\beta$ -arabinofuranosides have been reported and a few are mentioned below. For a more thorough discussion about  $\beta$ -arabinofuranosides please consult Imamura and Lowary’s review about chemical synthesis of furanose glycosides, who refer to reported methods up until 2011.<sup>130</sup> Furthermore the work by Thadke *et al.* and Liu *et al.* are worth mentioning.<sup>131,132</sup>

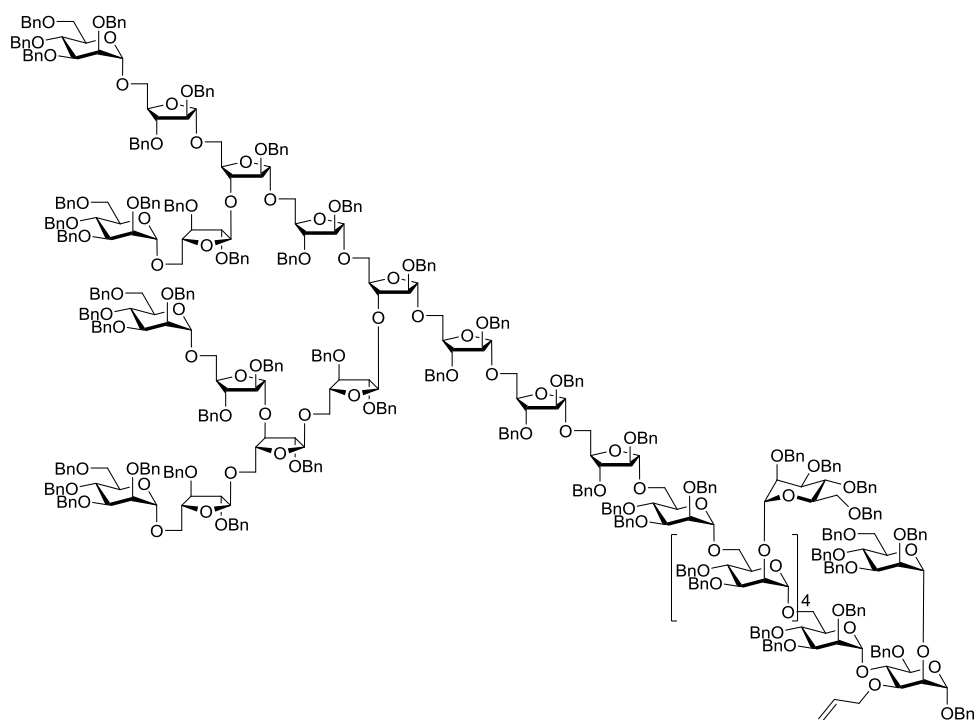
One of the first synthetic approaches to obtain  $\beta$ -arabinofuranosides were reported by D’Souza and Lowary. They succeeded in introducing both  $\beta$ -D-arabinofuranosyl residues from hexaarabinoside motif **2** (**E** and **F** from Figure 4) simultaneously in good yield and with excellent stereocontrol (Scheme 1).<sup>128</sup>

The authors suggest, that the stereoselectivity might result via a ion-pair  $S_N1$  pathway, but no studies following up on the selectivity of this monosaccharide was reported till recently.<sup>133</sup> These studies suggest that  $\beta$ -arabinofuranosidation is influenced by the stereochemical environment around the hydroxyl group of the acceptor and the temperature of the reaction. Low temperatures on sterically demanding and less reactive substrates afford better  $\beta$ -selectivity.



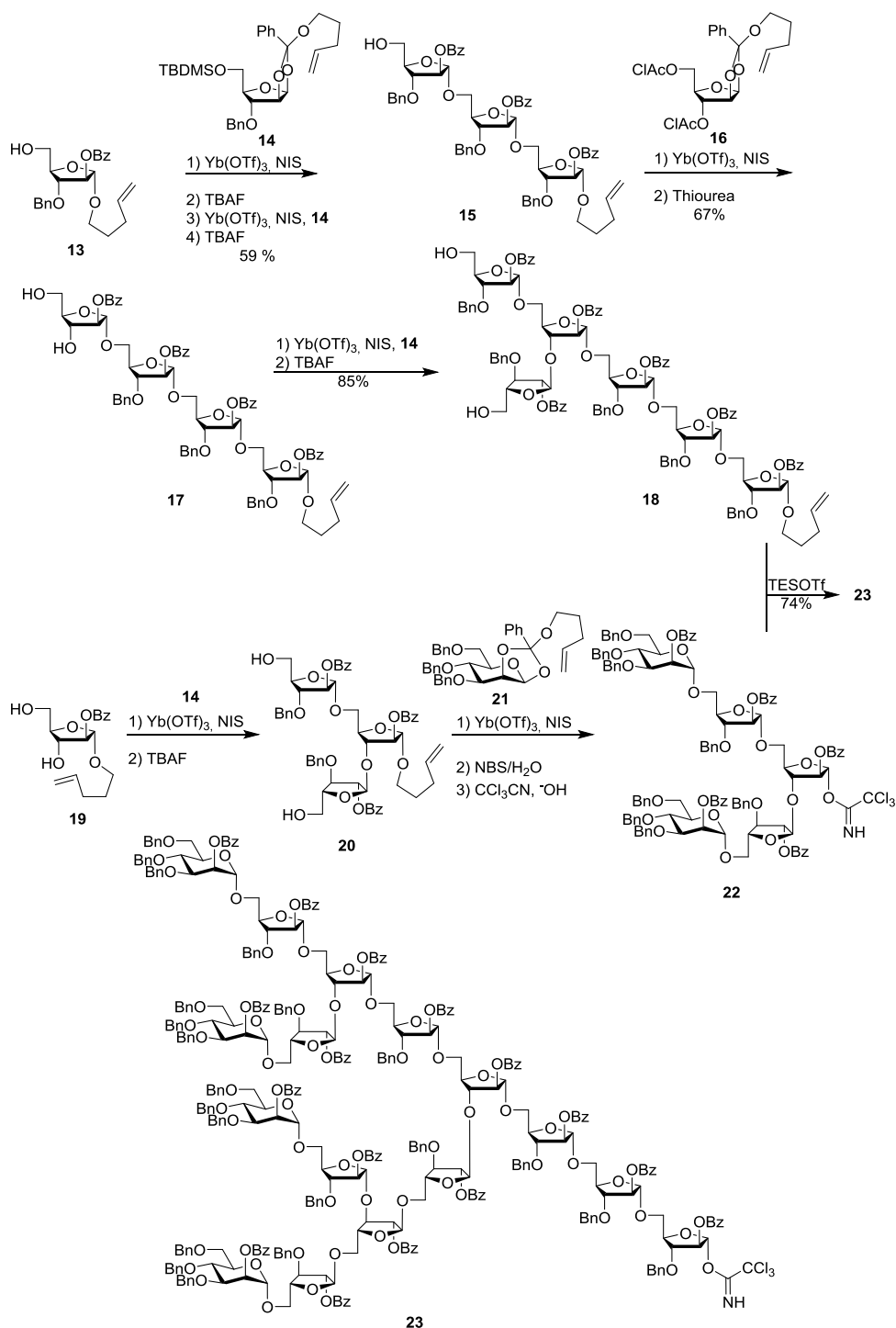
**Scheme 1** The first total synthesis of hexaarabinofuranoside **2**, with two  $\beta$ -linkages.<sup>128</sup>

In 2006, Fraser-Reid *et al.* synthesized an impressive 28-mer oligosaccharide core (**12**, Figure 5) of LAM using *n*-pentenyl orthoesters catalyzed by ytterbium triflate and promoted with NIS.<sup>134</sup> Trichloroacetimidate donors were used when the *n*-pentenyl orthoester donors did not perform satisfactory. At the time the oligosaccharide was prepared, it was the longest hetero-oligosaccharide synthesized, recently exceeded by a heparin-related [4]<sub>n</sub> 40-mer.<sup>135</sup> The final product (**12**) was the fully benzylated compound, and no attempts of deprotecting the oligosaccharide have been reported. The synthesis of the fully deprotected mannan core was reported in a different paper.<sup>136</sup> The linkage between the arabinoside and the mannoside in the synthesized oligosaccharide was a (1 $\rightarrow$ 6) connection, even though it other places is reported to be a (1 $\rightarrow$ 2) linkage.<sup>124</sup> The synthetic route to the mannanarabinan fragment (**23**) of oligo structure **12** is depicted in Scheme 2, (missing yields were not reported in the article).



12

**Figure 5** A 28-mer oligosaccharide synthesized by Fraser-Reid *et al.*<sup>134</sup>



Scheme 2 Synthetic route for the mannoarabinan fragment 23 of compound 12.<sup>134</sup>



The same year, Hölemann *et al.* reported a synthesis of the fully deprotected arabinomannan dodecaoligosaccharide (**24**, Figure 6).<sup>137</sup> The synthesis of the arabinan motif (Scheme 3), began with synthesis of the arabinan core from four building blocks **26-29**, all prepared from ortho ester **25**. They used trichloroacetimidate donors to obtain good glycosylation yields. To enable branching on the 3-position in building block **27**, they applied a levulinoyl protecting group, which could be removed selectively in the presence of the benzyl- and benzoyl groups using hydrazine monohydrate. With tetrasaccharide diol **35** in hand, they introduced another two arabinose residues simultaneously before transforming the hexasaccharide into trichloroacetimidate **36**. Hexasaccharide **36** was used as a donor in a [6+6] glycosylation with a mannohexaose acceptor before full deprotection to obtain compound **24**. Hexasaccharide **36** was also coupled with a 6-(benzylthio)-hexan-1-ol linker and thereafter fully deprotected to obtain hexasaccharide **37**. Another arabinohexasaccharide with two free secondary hydroxyl groups in the non-reducing end were also synthesized to introduce mannan cappings, however with limited success. This work by Hölemann *et al.* demonstrated a flexible synthetic strategy involving late-stage couplings and modifications, without addressing the more challenging  $\beta$ -linkages in the target molecules.

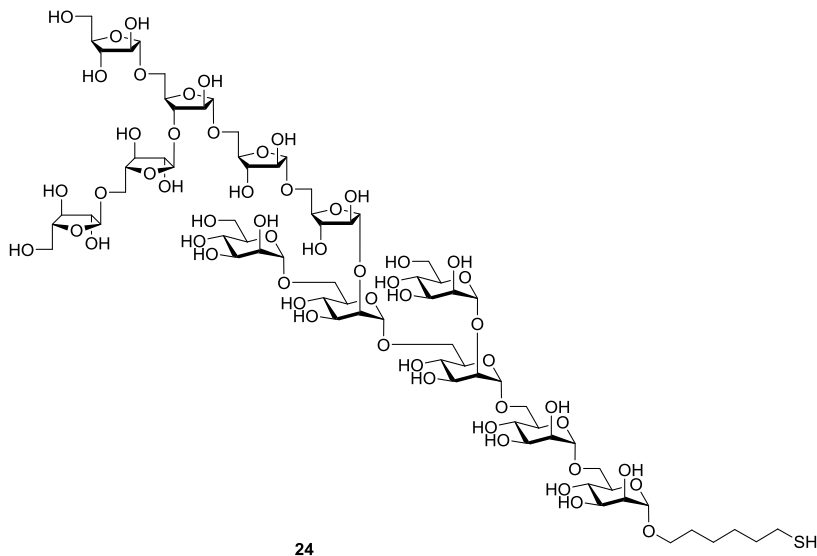
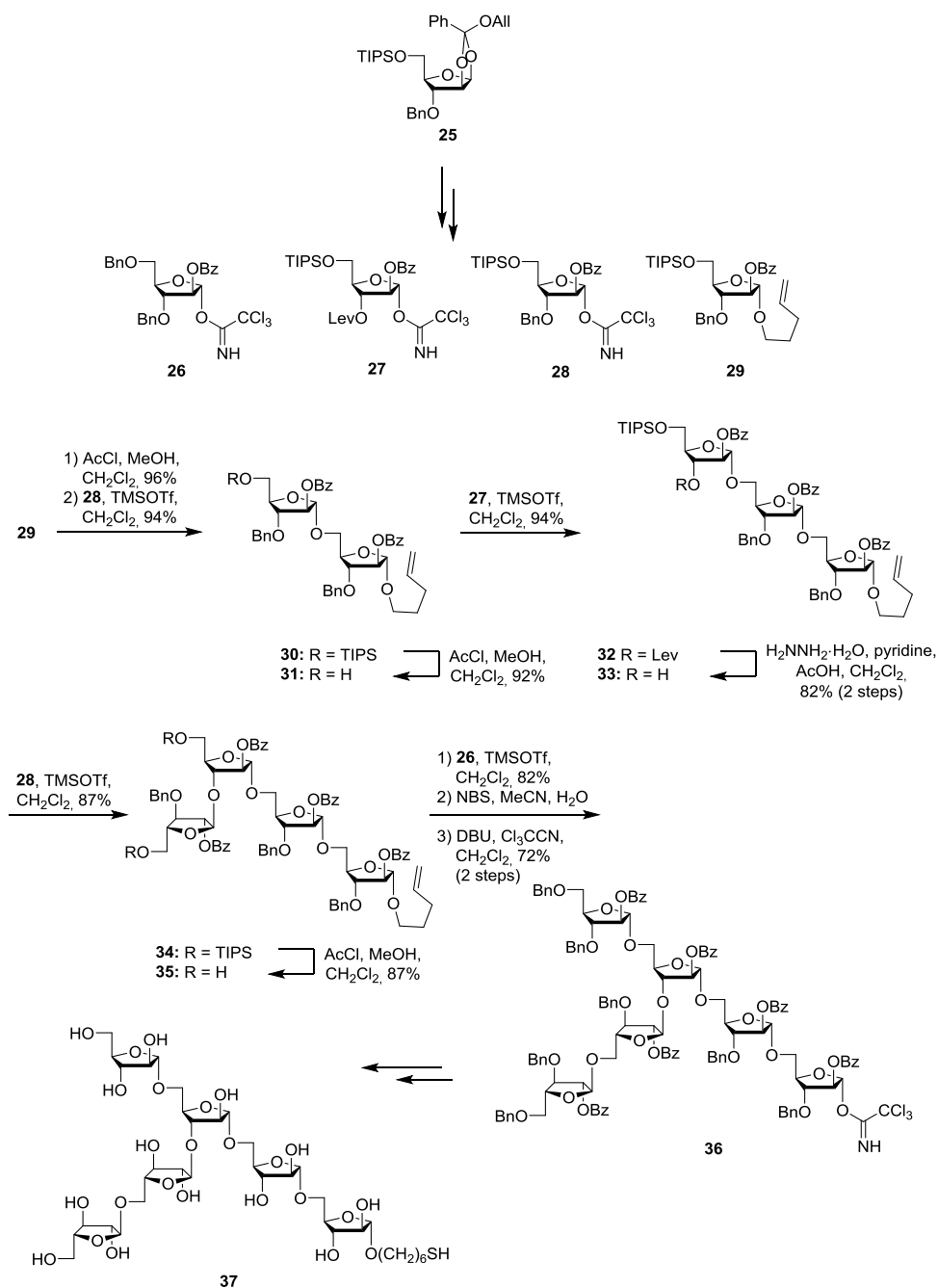
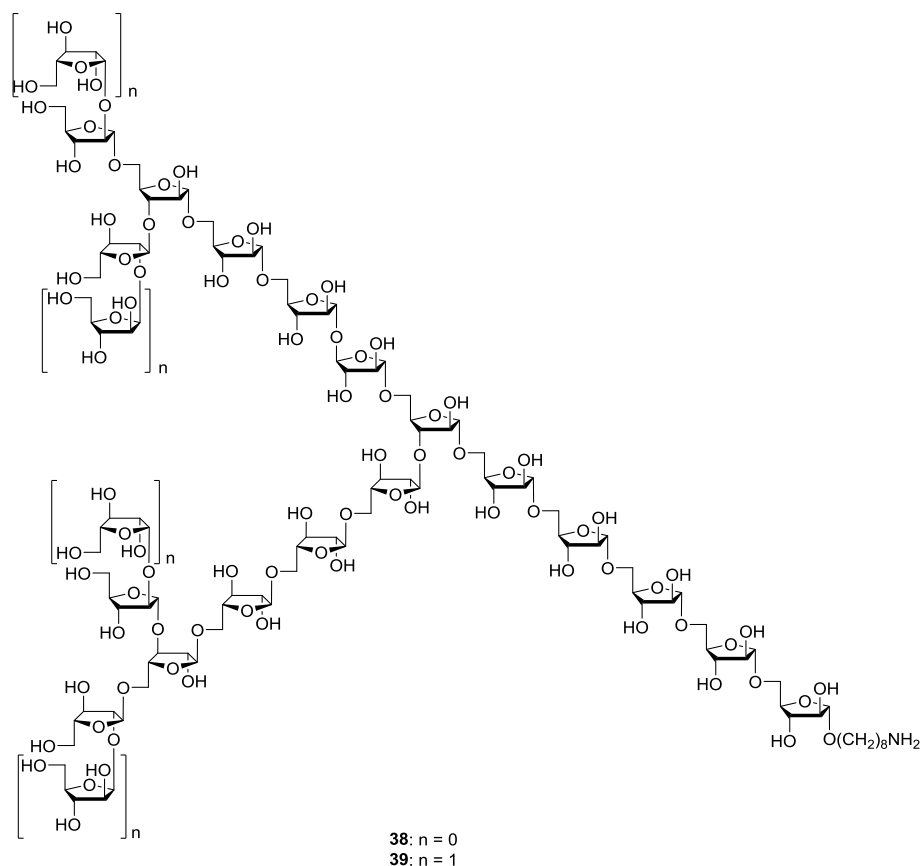


Figure 6 Arabinomannan synthesized by Hölemann, Stocker, and Seeberger.<sup>137</sup>

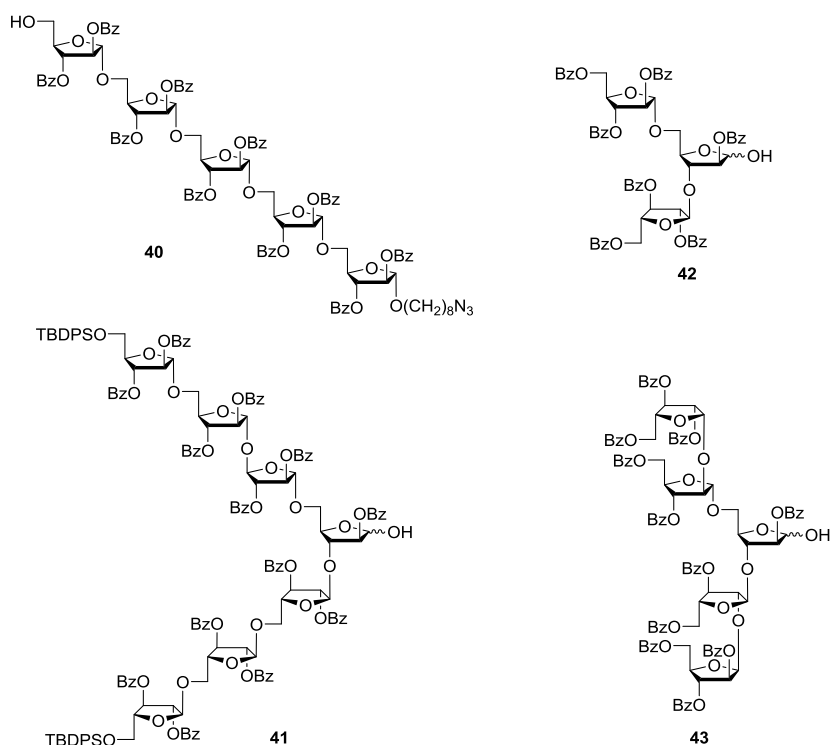
Scheme 3 Synthesis of the arabinan motif of compound **24**.<sup>137</sup>

Joe *et al.* reported a synthesis of an octadeca- and a docosanasaccharide (**38** and **39**, Figure 7).<sup>138</sup> The 22-mer includes four  $\beta$ -residues. A different synthetic route to the same oligosaccharide was reported in 2011 by Ishiwata and Ito.<sup>139</sup>



**Figure 7** Octadeca- and docosanaarabinofuranoside synthesized by Joe *et al.*<sup>138</sup>

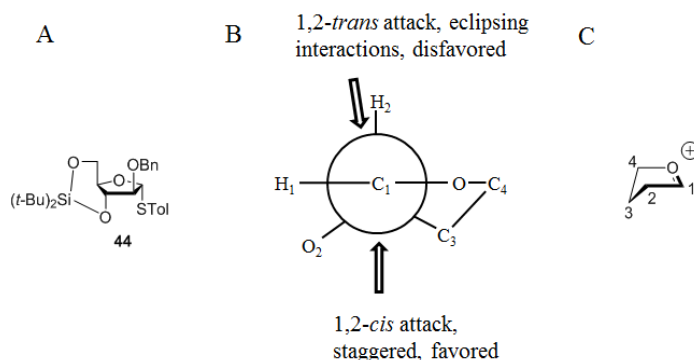
A key part of the strategy was to use building blocks protected only with benzoyl groups to ease the final deprotections. Tri- to heptasaccharide building blocks (**40-43**, Figure 8) were convergently glycosylated in sequential couplings to obtain the two target molecules. A combination of trichloroacetimidate and thioglycoside donors was used to afford these four building blocks. The symmetric nature of building blocks **41-43** made it possible to perform double glycosylations. The number of glycosylations was kept down using a disaccharide donor to elongate the chain in both compound **40** and compound **41**.



**Figure 8** Building blocks required for the synthesis of compound 38 and 39.

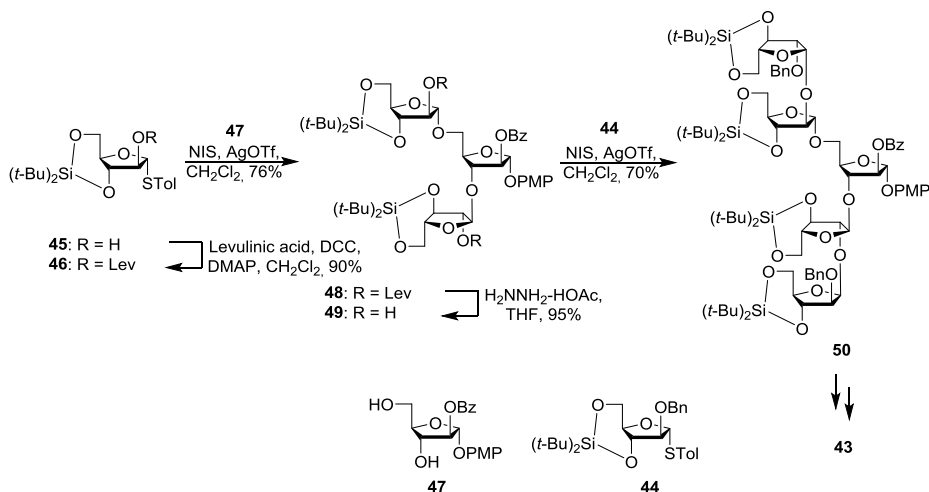
The introduction of the key  $\beta$ -arabinofuranoside residues in building block **43** (Figure 8) relied on a conformationally rigidified thioglycoside donor (**44**, Figure 9A), which favored attack from the  $\beta$ -face, a method first reported by Zhu *et al.*<sup>140</sup> A study of the low energy conformers of the arabinofuranoside oxa-carbenium ion (B) indicated that the E<sub>3</sub> conformer (Figure 9C), would favor approach from the  $\beta$ -face, since only staggered substituents would be experienced, whereas attack from the  $\alpha$ -face should be disfavored due to eclipsed H-2.<sup>1</sup>

<sup>1</sup> As opposed to pyranosides, which primarily exist in the <sup>4</sup>C<sub>1</sub> chair conformation and occasionally in the <sup>1</sup>C<sub>4</sub> or the boat conformation, five membered rings can exist in many different conformations. Either they are found as an envelope conformation, in which four atoms lie in a plane and the last atom is displaced from this plane. As all five atoms can be displaced either up or down, this gives rise to ten different envelope structures. Alternatively it can exist as a twist conformation in which two atoms are displaced from the plane – one up and one down. Here are likewise ten different possible structures. The conformations are shown on the pseudorotational wheel in Appendix A.



**Figure 9** A) Structure of compound **40**. B) Optimized geometries of the  $E_3$  conformers of the oxacarbenium ion of 2,3,5-tri-*O*-methyl-L-arabinofuranoside. C) The  $E_3$  conformation.

First glycosylation between thioglycoside donor **46** and diol acceptor **47** afforded trisaccharide **48**, then the two levulinate esters protecting the 2-positions were cleaved to afford a new diol acceptor **49** to which the  $\beta$ -arabinofuranoside residues were introduced via thioglycoside donor **44** in 70% yield. Manipulation of protecting groups gave building block **43**.



**Scheme 4** Synthesis of building block **43**.

The reducing end pentasaccharide **40** was coupled with a trichloroacetimidate donor derived from heptasaccharide **41** to give the reducing end dodecasaccharide for both compound **38** and **39**. By cleaving the two TBDPS groups in the non-reducing end, a diol was obtained, which again was coupled with the trichloroacetimidate donors prepared from either trisaccharide **42** or





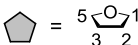

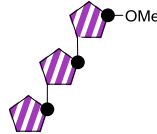

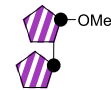
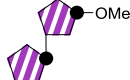
pentasaccharide **43** in a double glycosylation. Treatment with base followed by reduction afforded target molecules **38** and **39**.

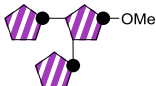

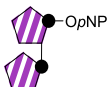
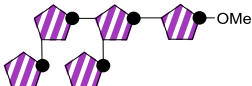
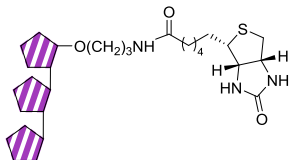
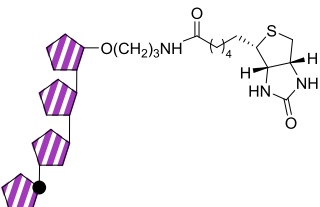
### 1.4.2 Chemical synthesis of oligo L-arabinofuranosides

So far not many synthesized oligo-L-arabinofuranosides have been reported, and literature still lacks examples of any fully unprotected targets. All reported examples minimum carry a protecting group on the anomeric position of the reducing end glycoside.

The following serve, to the best of our knowledge, as a comprehensive resume of reported synthesized oligo-L-arabinofuranosides. An overview of the synthesized structures is presented in Table 2. When it comes to manipulation of protecting groups on mono-L-arabinans, the literature holds more examples, but it is not considered relevant to mention them all in this context. Neither are the reported syntheses of oligosaccharides with substituents of monosaccharide L-arabinofuranosides, such as arabinoxylans and arabonogalactans.

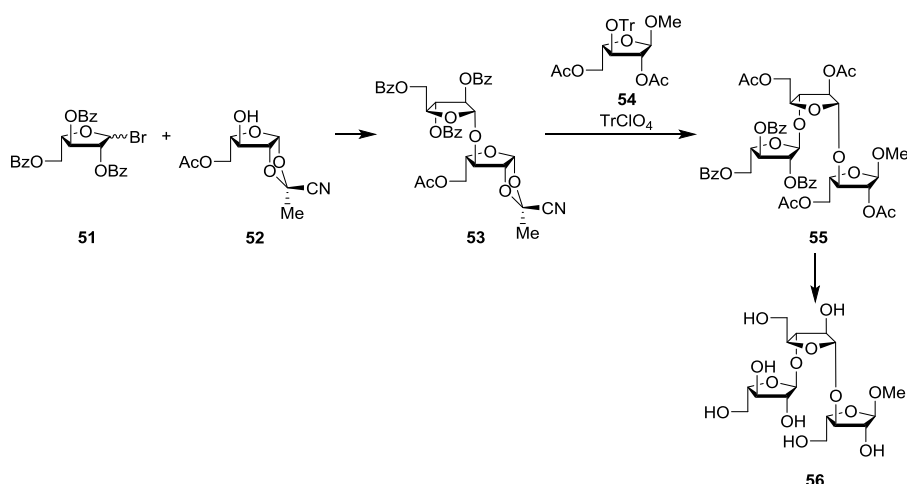
**Table 2 Overview of reported synthesized oligo L-arabinofuranosides.**

Entry	Structure	Reference
Legend		
	 L-Enantiomer  β-notation  D-Arabinose  α-notation  = $\begin{matrix} 5 & \text{O} & 1 \\ & \diagdown & / \\ & 3 & 2 \end{matrix}$	
1		Arndt and Graffi 1976 <sup>141</sup>
2		Nepogodev <i>et al.</i> 1986 <sup>142</sup>
3		Kawabata <i>et al.</i> 1995 <sup>143</sup>
4		Kawabata <i>et al.</i> 1995 <sup>143</sup>
5		Kawabata <i>et al.</i> 1995 <sup>143</sup>

6		Kaneko <i>et al.</i> 1995 <sup>144</sup>
7		Du <i>et al.</i> 1999; Du <i>et al.</i> 2000 <sup>145,146</sup>
8	 Transglycosylation product	Rémond <i>et al.</i> 2004; Chlubnová <i>et al.</i> 2010 <sup>147,148</sup>
9	 OBS. Fully protected	Deng <i>et al.</i> 2012 <sup>149</sup>
10		Kaeothip and Boons 2013 <sup>150</sup>
11		Kaeothip and Boons 2013 <sup>150</sup>

In 1976 Arndt and Graffi presented the synthesis of an (1→5) linked  $\alpha$ -L-diarabinofuranoside capped with a *p*-nitrophenyl group at the reducing end of the glycon (Table 2, entry 1).<sup>141</sup> The disaccharide was prepared from a perbenzoylated bromide donor and a 5-*O*-trityl 2,3-di-*O*-acetylated *p*-nitrophenyl acceptor in 25% yield.

To investigate a polymerization reaction Nepogodev *et al.* first reported the synthesis of a linear (1→3)- $\alpha$ -L-trisaccharide (Table 2, entry 2).<sup>142</sup> By coupling disaccharide **53** (Scheme 5) with monosaccharide **54** in the presence of  $\text{TrClO}_4$  they synthesized trisaccharide **55**, which was deprotected to obtain the unprotected trisaccharide **56**, capped with an anomeric methyl group.

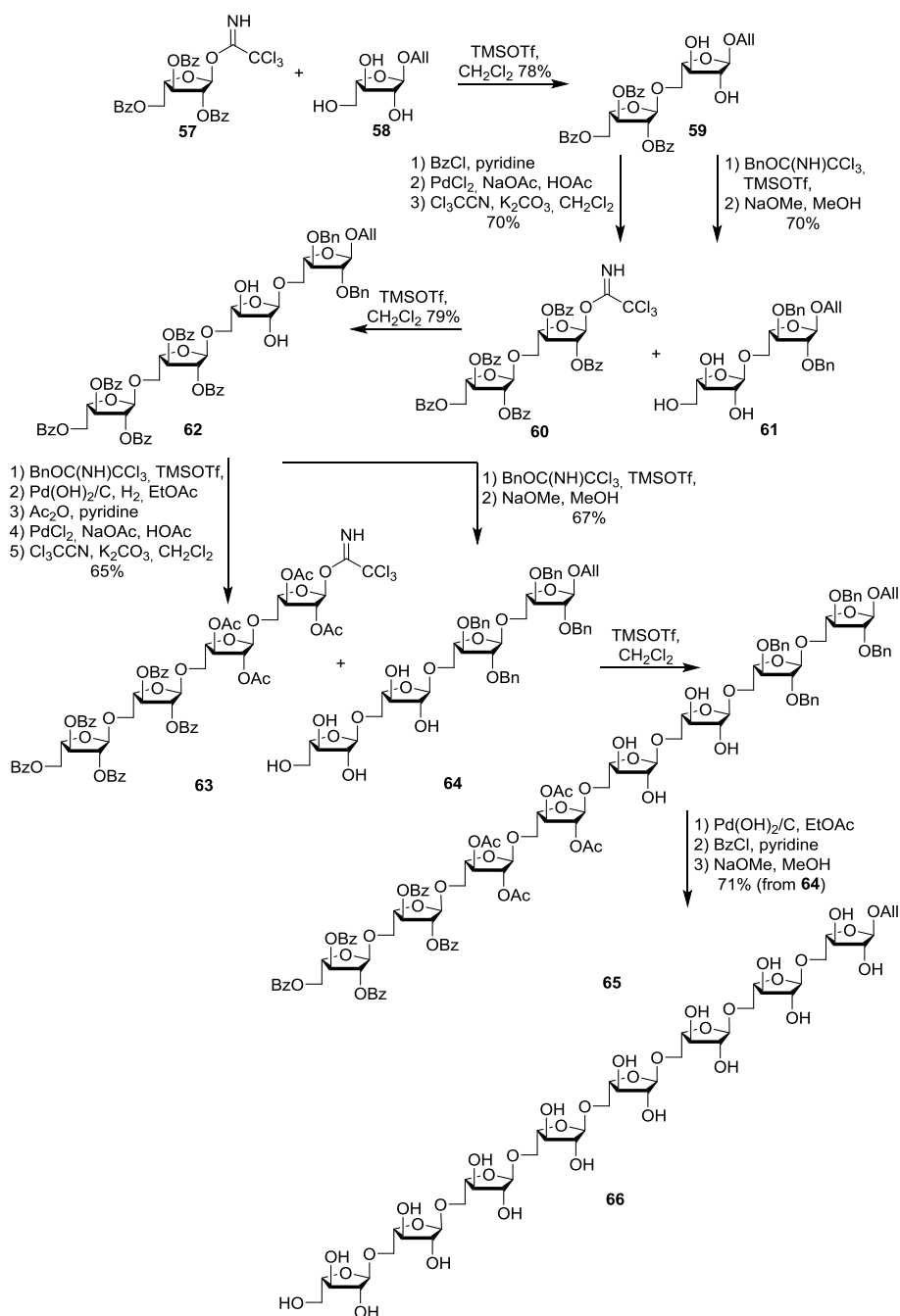


**Scheme 5** Synthesis of a linear (1→3)-α-L-trisaccharide.<sup>142</sup>

Kusakabe *et al.* synthesized all three α-L-diarabinofuranosides and the trisaccharide shown in Table 2, entry 3-6, all synthesized with methanol as reducing end aglycon, using Koenigs-Knorr glycosylations promoted by AgOTf and *sym*-collidine.<sup>143,144</sup> The synthesis of the trisaccharide involved simultaneous couplings of the 3- and the 5-position of the reducing end monosaccharide. Methyl glycosides were chosen as targets molecules for enzymatic studies, because the methyl-glycosidic linkage is largely resistant to α-L-arabinofuranosidases. As an extra benefit, methyl glycosides return NMR spectra, which are easier to assign than those from “reducing sugars” with a free hydroxyl group in the reducing end.

Kong *et al.* have synthesized a linear (1→5)-α-L-octaarabinosaccharide with an allyl group capping the reducing end of the molecule. (Table 2, entry 7).<sup>145,146</sup> They were able to perform a regioselective glycosylation between the perbenzoylated trichloroacetimidate donor **57** and unprotected allyl α-L-arabinofuranoside **58** to obtain partly protected disaccharide **59** in 78% yield (Scheme 6). The disaccharide was converted to a disaccharide donor (**60**) and a triol disaccharide acceptor (**61**). The glycosylation between the two also proceeded in good yield (79%). The tetrasaccharide **62** was likewise converted into a tetrasaccharide donor **63** and a tetrasaccharide acceptor **64**, holding five free hydroxyl groups. No yield was reported for the glycosylation, but the free octasaccharide **66** was obtained in 71% yield based on tetrasaccharide acceptor **64**.<sup>145</sup> The same octasaccharide was synthesized from a disaccharide trichloroacetimidate donor, which selectively was coupled to the 5-position of unprotected allyl α-L-arabinofuranosides with five free hydroxyl groups, under





**Scheme 6** Synthesis of linear allyl octa (1→5)-α-L-arabinofuranoside **66**.<sup>145</sup>

the promotion of trimethylsilyl trifluoromethanesulfonate (TMSOTf).<sup>146</sup> It is worth mentioning that unprotected linear arabinans, prepared by controlled

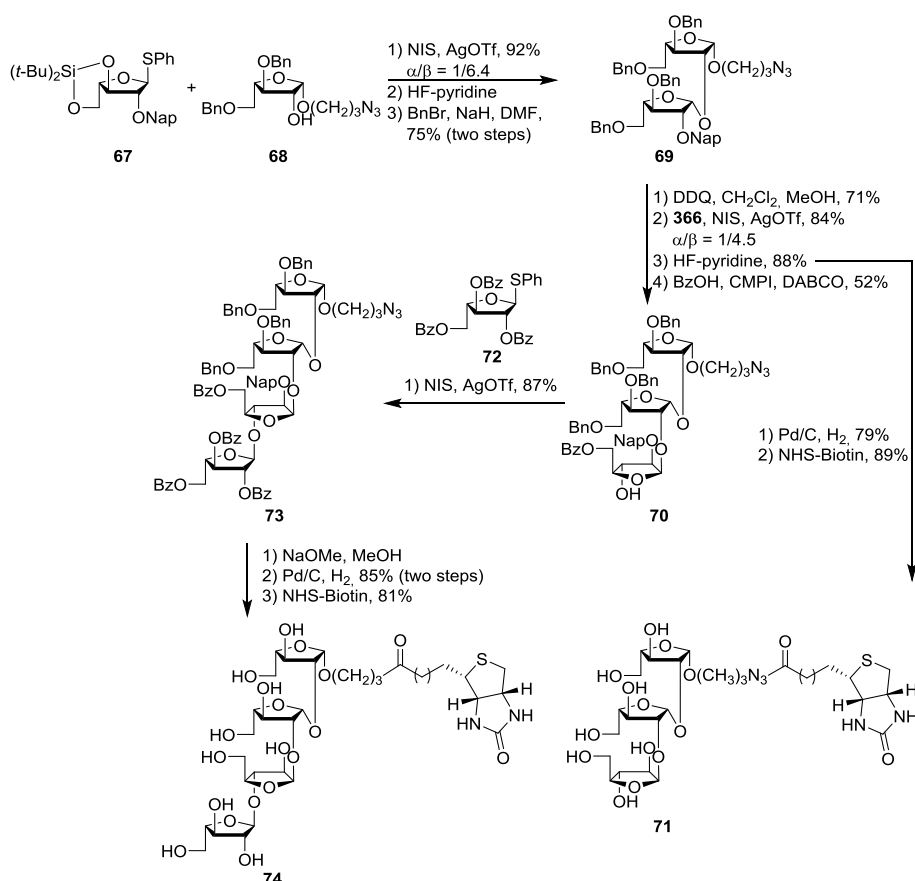
enzymatic hydrolysis of debranched sugar beet arabinan, are commercially available via Megazyme, up to the linear (1→5)- $\alpha$ -L-octasaccharide in more than 90% purity.

The synthesis of a tri- and a tetrasaccharide (1→2)- $\beta$ -L-arabinofuranoside (**71** and **74**, Scheme 7), with an *N*-hydroxysuccinimide ester (NHS-biotin) capping the reducing end of the glycon was recently presented by Kaeothip and Boons (Table 2, entry 10 and 11).<sup>150</sup> (1→2)- $\beta$ -L-Arabinofuranosides are found in plant cell wall extensins, which are plant derived glycoproteins that are densely modified by oligoarabinofuranosides linked to hydroproline residues.<sup>151</sup>

Using the conformationally strained thioglycoside donor **67** (Scheme 7), Kaeothip and Boons were able to get  $\beta$ -selectivity when glycosylating with acceptor **68**, like explained in relation to Scheme 4. The 2-Naphthylmethyl (Nap) group was cleaved from disaccharide **69** before another glycosylation with donor **67** afforded a trisaccharide in 84% yield with 1/4.5  $\alpha/\beta$  selectivity. The silyl ring and the benzoyl groups were removed and then the trisaccharide was reacted with NHS-Biotin to obtain target molecule **71**. The trisaccharide diol obtained after desilylation was selectively protected with a benzoyl group in the 5-position of the reducing end monosaccharide in 52% yield using benzoic acid in the presence of 2-chloro-1-methylpyridinium iodide (CMPI) and 1,4-diazabicyclo[2.2.2]octane (DABCO) to obtain acceptor **70**. The  $\alpha$ -moity capping the non-reducing end was installed using thioglycoside **72** to afford tetrasaccharide **73** in 87% yield. Cleaving the benzoyl and benzyl groups from compound **73** and reacting the tetrasaccharide with NHS-Biotin obtained target molecule **74**.

Deng *et al.* have synthesized a fully protected pentaarabinan, in a one-pot fashion composed of a trisaccharide substituted with an  $\alpha$ -L-arabinose unit in the 3-position of the two monosaccharides in the reducing end of the chain Table 2, entry 9.<sup>149</sup>

Besides the synthesis described in this section, L-arabinofuranosides are employed different places in larger molecules. Please consult the references for further examples.<sup>152,153</sup>



Scheme 7 Synthesis of a tri- and a tetrasaccharide (1→2)-β-L-arabinofuranoside.<sup>150</sup>

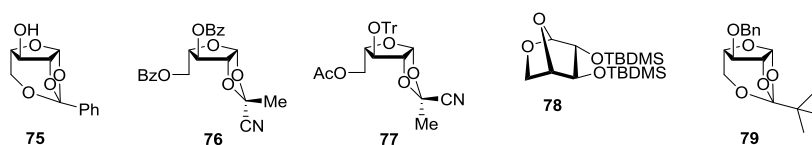
### 1.4.3 L-Arabinofuranosides obtained from polycondensation

Over the years several examples of polycondensations of different monosaccharides have been reported to produce oligo-L-arabinofuranosides. In 1969 Kochetkov *et al.* reported the polymerization of the tricyclic ortho-ester β-L-arabinofuranose 1,2,5-ortho-benzoate **75**, Figure 10 in nitromethane with catalytic amounts of mercuric bromide.<sup>154</sup> The synthesized polymer was a highly branched L-arabinofuranoside containing (1→5)-α- (and a few (1→3)-) linkages, with branch points at the 3-position with an average degree of polymerization  $\overline{DP} = 59.5$ , obtained in 50% yield. When the 3-*O*-acetyl protected **75** was polymerized in the presence of an initiator (1,2,3,4-tetra-*o*-acetyl-β-D-glucopyranose) the main product was an (1→5)-α-L-arabinofuranoside with a few (1→2) linkages, capped with a D-glucose residue in the reducing end of each chain.

In 1985 Backinowsky *et al.* reported a stereospecific polycondensation of monosaccharide **76** (Figure 10).<sup>155</sup> The obtained poly (1→5)- $\alpha$ -L-arabinofuranose only gave rise to five different signals in the  $^{13}\text{C}$  NMR spectrum, which the authors ascribes the high stereo- and regiospecificity associated with the polycondensation. However, oligoarabinofuranosides are most often reported with several peaks for at least C-1 and C-5,<sup>60,76,156</sup> since the chemical environment for C-5 differs from the non-reducing end to C-5's in the chain. Likewise the chemical environment for C-1 in the reducing end of the saccharide likewise varies from the C-1's in the rest of the oligosaccharide. However, in polysaccharides the ratio between the C-5 in the non-reducing end and the additional C-5's might suppress the first mentioned signal, so only the carbons in the homogenous chain are seen as one peak. Similarly a polycondensation of monosaccharide **77** in the presence of  $\text{TrClO}_4$  was reported the following year by the same authors.<sup>142</sup> The highest molecular weight fraction was a regular (1-3)- $\alpha$ -polymer with a DP of 40-45.

Using 1,4-anhydro-2,3-di-*O*-*tert*-butyldimethylsilyl- $\alpha$ -L-arabinopyranose **78**, as ringopening polymerization monosaccharide Yoshida *et al.*<sup>157</sup> obtained (1→5)- $\alpha$ -L-arabinofuranoside. However, this procedure didn't lead to well-defined arabinan.

Another polymerization producing stereoregular (1→5)- $\alpha$ -L-arabinofuranosides was reported by Hori and Nakatsubo.<sup>158</sup> In this case the monosaccharide was 3-*O*-benzyl- $\beta$ -L-arabinofuranose 1,2,5-orthopivalate **79** (Figure 10), which resulted in a polysaccharide backbone, in which it was possible to distinguish between *O*-2 and *O*-3, due to different protecting groups.



**Figure 10** Monosaccharides used in polycondensation reactions to obtain L-arabinofuranosides.

#### 1.4.4 L-Arabinofuranosides obtained from transglycosylations

Increasingly, enzymes are adopted for the synthesis of oligosaccharides. The first enzymatic approach for synthesis of arabinofuranosides was reported in 2004.<sup>147</sup> The thermostable arabinosidase from *Thermobacillus xylanilyticus* was able to catalyze transglycosylation in the presence of *p*-nitrophenyl  $\alpha$ -L-arabinofuranoside to obtain *p*-nitrophenyl  $\alpha$ -L-arabinofuranosyl

(1→2)- $\alpha$ -L-arabinofuranoside in 60-70% yield (Table 2, entry 8), along with small quantities of the (1→3) and (1→5) linked disaccharides. This enzyme was also found to catalyze transglycosylation of *p*-nitrophenyl  $\beta$ -D-xylopyranoside to obtain the (1→2) and (1→3) linked disaccharides, but to a lesser extent. The enzyme did not show activity towards *p*-nitrophenyl  $\alpha$ -L-arabinopyranoside and *p*-nitrophenyl  $\beta$ -D-xylofuranoside.

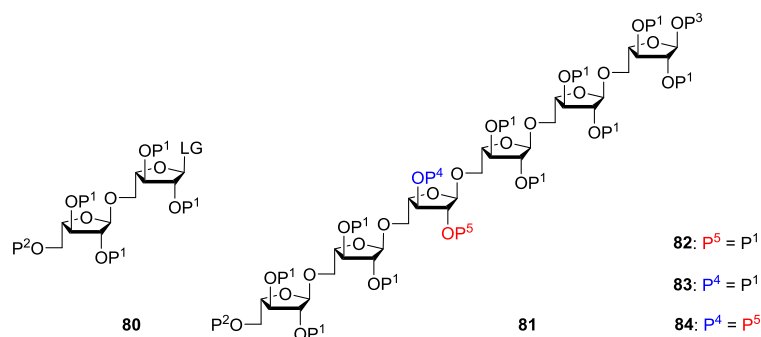
Another arabinofuranosidase from *Clostridium thermocellum* was studied by Chlubnova *et al.*<sup>148</sup> This enzyme also catalyzed the transglycosylation of *p*-nitrophenyl  $\alpha$ -L-arabinofuranoside to synthesize the (1→2)- $\alpha$ -L disaccharide (Table 2, entry 8) as the major product in 13% yield.

## 2 Strategy and challenges

The aim of this project was to synthesize (1→5)  $\alpha$ -L-arabinofuranosides branched in either the 2- or the 3-position mimicking the branched structure of the RG-I region of pectin to obtain a better biochemical understanding hereof.

We wished to design a synthetic strategy, in which the branching was introduced at a late stage in the synthesis, which would enable us to introduce various branchings on the same backbone structure thereby getting hands on a variety of oligosaccharide structures by changing the late-stage synthesis strategy.

For the synthesis of the backbone structure, we were encouraged by the convergent strategy used by Joe *et al.*<sup>138</sup> utilizing a disaccharide donor (**80**, Figure 11), which held an orthogonal protecting group ( $P^2$ ) in the 5'-position, enabling elongation of the chain. We aimed for a hexasaccharide backbone (**81**) with a  $P^3$  protecting group, of non-leaving group nature, protecting the anomeric hydroxyl group. The hexasaccharide structure should hold branching possibilities on the fourth monosaccharide. Ideally only one backbone structure holding two orthogonal protecting groups ( $P^4$  and  $P^5$ ) was needed, however, it was very challenging to find so many different protecting groups, all being truly orthogonal. Therefore it was decided to synthesize three different hexasaccharide backbone structures (**82**, **83** and **84**), holding three different monosaccharide substituents as the fourth monosaccharide. In octasaccharide **82**  $P^5 = P^1$ , enabling branching on the 3'''-position, whereas branching was made possible in the 2'''-position in compound **83**, where  $P^4 = P^1$ , and simultaneous branching was feasible in decasaccharide **84**, where  $P^4 = P^5 \neq P^1$ .



**Figure 11** Illustration of disaccharide donor **80** and hexasaccharide backbone structures **81-84**.

As previous work on arabinofuranosides primarily was aimed towards linear or branched oligosaccharides, in which the branching and the continuation of the chain were introduced simultaneously, we wished to develop a synthesis of three monosaccharide building blocks with an orthogonal protecting group in the 2- or the 3-position or in both positions.

We decided primarily to use thioglycosides as glycosyl donors, as there was experience with this donor in the group. This type of donor is widely used, because of its appreciated stability towards many chemical manipulations and its readily activation with a variety of thiophilic reagents.<sup>159</sup> The many advantages of thioglycosides have recently been reviewed by Lian *et al.*<sup>160</sup> In this work, the synthesis presented have mainly focused on *N*-iodosuccinimide (NIS)/silver triflate or triflic acid (TfOH) as activator systems. Additionally, a few experiments were conducted with the widely used trichloroacetimidates and *N*-phenyl trifluoroacetimidates, developed by Schmidt and co-workers<sup>161</sup> and Yu and Tao, respectively.<sup>162</sup>

The target molecules in this work are  $\alpha$ -arabinofuranosides; five-membered rings of arabinose connected by  $\alpha$ -linkages. Fortunately the  $\alpha$ -compounds are the least challenging to synthesize, as discussed in relation to  $\beta$ -arabinofuranosides previously. The furanoside structures are either obtained via the Fischer glycosylation,<sup>163</sup> which affords the furanoside as the kinetic product, or by reacting arabinose with a silyl protecting group with preference for primary hydroxyl groups.

## 2.1 NMR characterization

All the synthesized compounds presented in this thesis were analyzed by NMR. Except for a few, all 1D  $^1\text{H}$  and  $^{13}\text{C}$  spectra have been fully assigned by the use of the 2D spectra: heteronuclear single quantum coherence (HSQC), HMBC and double quantum filtered correlation spectroscopy (DQF-COSY).

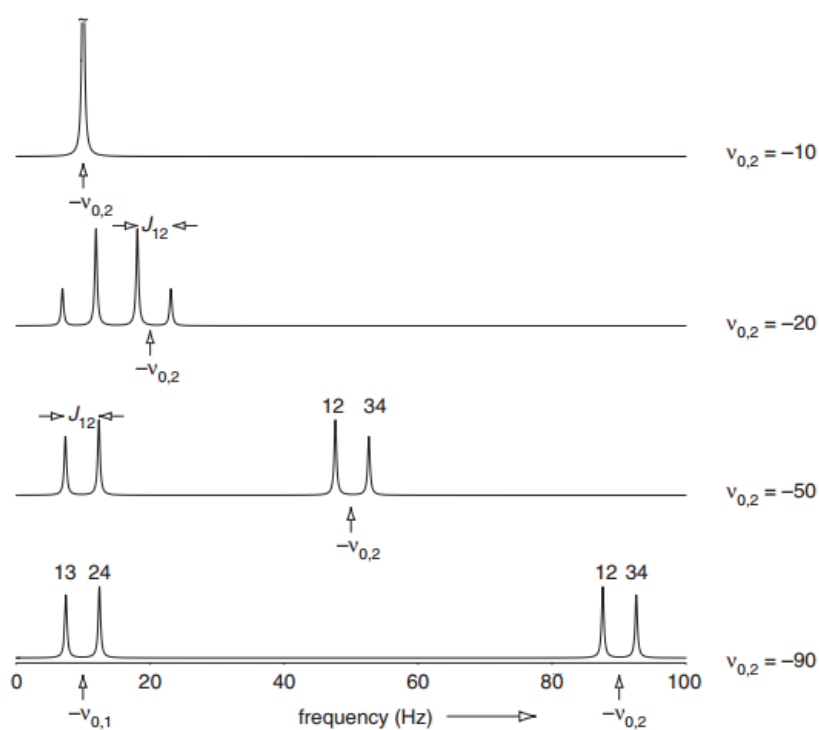
In carbohydrate chemistry it is important to verify, if the obtained structure is the  $\alpha$ - or the  $\beta$ -anomer. For fully deprotected structures the anomeric carbons of  $\alpha$ -arabinofuranosides resonate in the range of 105-110 ppm, and the  $\beta$ -anomers appear between 97 and 104 ppm.<sup>164,165</sup> In addition,  $^3J_{\text{H1,H2}}$  is small (0-2 Hz) for the  $\alpha$ -anomers and larger (4-5 Hz) for the  $\beta$ -anomers. The same tendency is true for the protected mono- and oligosaccharides, where C-1  $\alpha$ -carbons resonate further downfield than  $\beta$ -carbons, as opposed to e.g. glucopyranosides, in which the shift of the anomeric  $\beta$ -carbon lies further downfield than the one for the  $\alpha$ -carbon. The carbon shift, however, also depends on the functional group

protecting the anomeric hydroxyl group. The anomeric carbons in  $\alpha$ -thioglycosides give rise to peaks around 90 ppm, whereas the anomeric carbon in  $\alpha$ -compounds, with an ester group protecting the hydroxyl group, resonates close to 100 ppm, and with an ether group it resonates around 105 ppm. The C-1s in the chain of (1 $\rightarrow$ 5)- $\alpha$ -arabinofuranosides have frequencies between 105 and 108 ppm. In the 1D  $^1\text{H}$  spectrum, protons deshielded by neighboring hydroxyl protecting groups are found further downfield than those in unprotected carbohydrates. Generally H-1 to H-3 adjacent to protected hydroxyl groups are found in the range of 5.0 - 6.0 ppm, whereas H-4 is found between 4.4 and 4.8 ppm, and the two H-5 protons are found around 3.7 to 4.2 ppm. The protons in the methylene group of benzyl arabinofuranosides are diastereoisotopic and therefore always split into two doublets around 4.85 and 4.60 ppm with a carbon chemical shift around 68.7 ppm for the synthesized compounds. The upfield  $^1\text{H}$  doublet often overlaps with H-4, whereas the downfield doublet of benzyl arabinofuranosides usually is separated from other signals, often as one of the only protons in the case of bigger oligosaccharides, why it can be used to normalize the integrals from.

The methylene group in the chloroacetyl group, which is used as an orthogonal protecting group throughout the synthesis, sometimes gives rise to a singlet integrating to two protons and are other times found as two doublets with a coupling constant of  $J = 15$  Hz. The pattern of the  $\text{CH}_2$  peak relates to the conformation of the molecule and how much the chloroacetyl group in that conformation is affected by the stereochemistry of the sugar moiety. Sometimes the two doublets are positioned so close to one another, that they give rise to an AB spin system.

AB spin systems are the result of strong coupling (or second order effects), occurring when the frequency difference between spins A and B approach the magnitude of  $J$ , and for this reason it is also observed more frequently at lower field strengths. Figure 12 shows an AB spin system in which the two protons resonate with increasing differences in frequency. Because of the asymmetry of the individual doublets, the so-called roof effect, the chemical shift value for each proton is not exactly in the geometrical center of the doublet, but rather closer to the tallest peak. It is therefore necessary to calculate the correct chemical shifts, which are also marked between the peaks on Figure 12, using the formulas in Figure 13.





**Figure 12** A series of spectra of a two spin system in which the frequency of spin 2 is moved closer to spin 1 (from bottom to top). The spectra become more and more strongly coupled, showing a pronounced roof effect until the two frequencies are equal and give rise to a singlet.<sup>166</sup>

1. Determine the four line positions in Hz, and measure  $J_{AB}$

$$|J_{AB}| = (v_1 - v_2) = (v_3 - v_4) = 10 \text{ Hz}$$

2. Calculate the center position (in Hz):

$$v_{center} = \frac{1}{2}(v_2 + v_3) = 2024.1$$

3. Calculate  $v_{AB}$ .

$$\Delta v_{AB} = \sqrt{(v_1 - v_4)(v_2 - v_3)} = 9.94 \text{ Hz}$$

4. Calculate  $v_A$  and  $v_B$  (spectrometer frequency: 300 MHz).

$$v_A = v_{center} + \frac{1}{2}\Delta v_{AB} = 2029.1 \text{ Hz}$$

$$v_B = v_{center} - \frac{1}{2}\Delta v_{AB} = 2019.1 \text{ Hz}$$

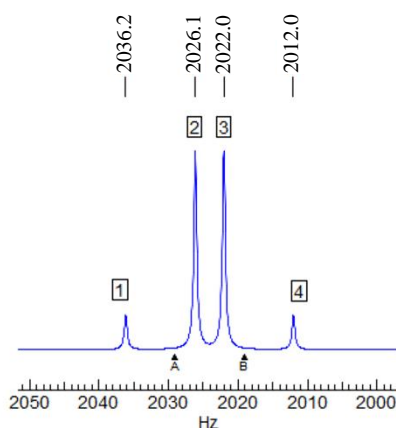
$$\delta_A = \frac{v_A}{\text{MHz}} = 6.76$$

$$\delta_B = \frac{v_B}{\text{MHz}} = 6.73$$

5. Combining the formulas

$$\delta_A = \frac{v_{center} + \frac{1}{2}\sqrt{(v_1 - v_4)(v_2 - v_3)}}{\text{MHz}}$$

$$\delta_B = \frac{v_{center} - \frac{1}{2}\sqrt{(v_1 - v_4)(v_2 - v_3)}}{\text{MHz}}$$

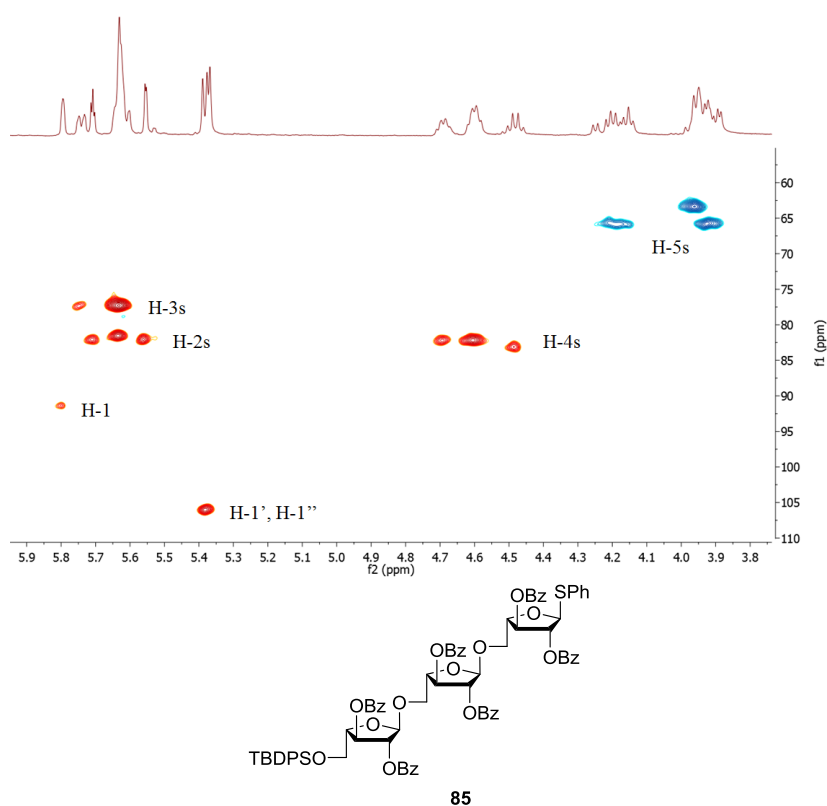


**Figure 13** Formulas used to calculate the actual peak for an AB spin system.

In case of the monosaccharides, it was easy to distinguish all the peaks from one another in the  $^{13}\text{C}$  spectra and assign them. The C-2s and C-4s resonate around 82 ppm and C-3s give rise to peaks very close to the solvent peak for

$\text{CDCl}_3$  around 77 ppm. The C-5s are found around 62 ppm. In case of oligosaccharides, the C-5 in the non-reducing end of the oligosaccharide is always found further upfield.

For the synthesized di- and trisaccharides, it was also possible to assign all peaks, even though the chemical environment for all C-2s was very similar, likewise the respective chemical environments for all C-3s and all C-4s were very alike, and all the peaks from one position were very closely spaced. As an additional challenge the C-2 and C-4 all resonated around 82 ppm. This often resulted in overlapping signals. By zooming in, it was possible to assign the separate peaks. An example of a HSQC spectrum of a trisaccharide is shown in Figure 14.



**Figure 14** HSQC spectrum of trisaccharide 85.

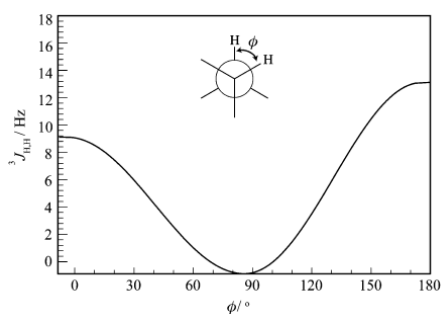
As the oligosaccharides got bigger, the spectral overlap increased and full NMR characterization became increasingly challenging working with datasets acquired in automation. With up to ten different protons in a 0.07 ppm interval, the demands for higher resolution increased.

Almost all the  $^3J_{\text{H1,H2}}$  in  $\alpha$ -arabinofuranosides are very close to 0 Hz, which results in missing cross peaks in the COSY spectra between H-1 and H-2, so even though the H-1s very often are well dispersed, they cannot be correlated to the H-2's using a COSY spectrum. The H2BC experiment<sup>167</sup> is sometimes used in the analysis of carbohydrate molecules, as the resulting spectrum almost exclusively shows correlations between proton and carbon spins separated by two covalent bonds. However no H2BC spectra of the compounds were run, and it probably would not have helped in this case anyway, since the H2BC correlation relies on  $^1J_{\text{CH}}$  and  $^nJ_{\text{HH}}$ , and since no  $^3J_{\text{H1,H2}}$  is present, probably no cross peak would have been seen in the H2BC spectrum.

The small coupling constant between H-1 and H-2 in  $\alpha$ -arabinofuranosides tells us something about the conformation of the molecule. The Karplus equation describes the correlation between  $^3J$  coupling constants and dihedral torsion angles in NMR:<sup>168</sup>

$$^3J_{n,n'} = A \cos^2 \varphi + B \cos \varphi + C$$

where  $^3J$  is the three-bond (homonuclear) coupling constant,  $\varphi$  is the dihedral angle, and A, B and C are empirically derived parameters whose values depend on the atoms and substituents involved. The  $^3J_{\text{H,H}}$  coupling constants are generally smallest when the torsion angle between the two protons is close to  $90^\circ$  and largest at angles of  $0^\circ$  and  $180^\circ$ , as illustrated by the Karplus curve in Figure 15. This tells us, that the torsion angle between H-1 and H-2 in most  $\alpha$ -arabinofuranosides is close to  $90^\circ$ .



**Figure 15 Karplus curve.**<sup>166</sup>

H-4 occasionally gives rise to a peak which resembles a quartet but in which the individual distances between the four peaks are not the same, see Figure 16 for three examples from compound  $\beta$ -**86**, **87** and **88**. In the first case the coupling constants obtained from the H-3 and H-5 cross peaks with H-4 are  $J_{\text{H3-H4}} =$

4.0 Hz,  $J_{\text{H4-H5a}} = 2.9$  Hz, and  $J_{\text{H4-H5b}} = 2.4$  Hz, which theoretically should give rise to a ddd. However, since  $J_{\text{H4-H5a}} \sim J_{\text{H4-H5b}}$ , H-4 appears as a doublet of triplets (dt), which can be recognized. In the next case  $J_{\text{H3-H4}} = 5.4$  Hz and  $J_{\text{H4-H5a+b}} = 4.2$  Hz, therefore H-4 should also be a dt, as is also recognized. But since it is very hard to read the coupling constants, it was chosen to report both the peaks as multiplets.

The two peaks shown for compound **88** are H-4' and H-4'', in these cases it is easier to recognize the coupling pattern as td and dt, which they were reported as with coupling constants  $J = 4.6$  Hz and 2.9 Hz for the first peak being a td and  $J = 5.0$  Hz and 3.6 Hz for the second peak being a dt.

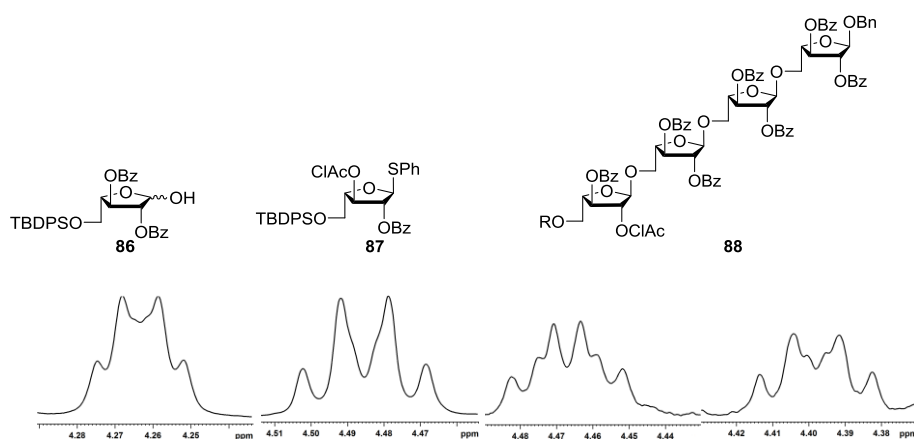


Figure 16 Peak structure of H-4 in compounds 86 and 87 88.

The two H-5 protons sometimes show as a doublet integrating to two protons, other times as an AB system of two doublet of doublets each integrating to one proton, which depends on whether the H-5s have different chemical shifts or not, which again depends on the local shielding environment of the oligosaccharide, Figure 17.

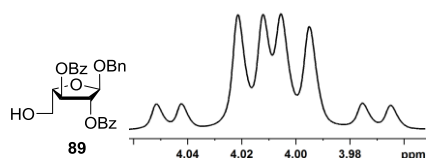


Figure 17 Peak structure of two doublet of doublets assigned to H-5a and H-5b in compound 89.

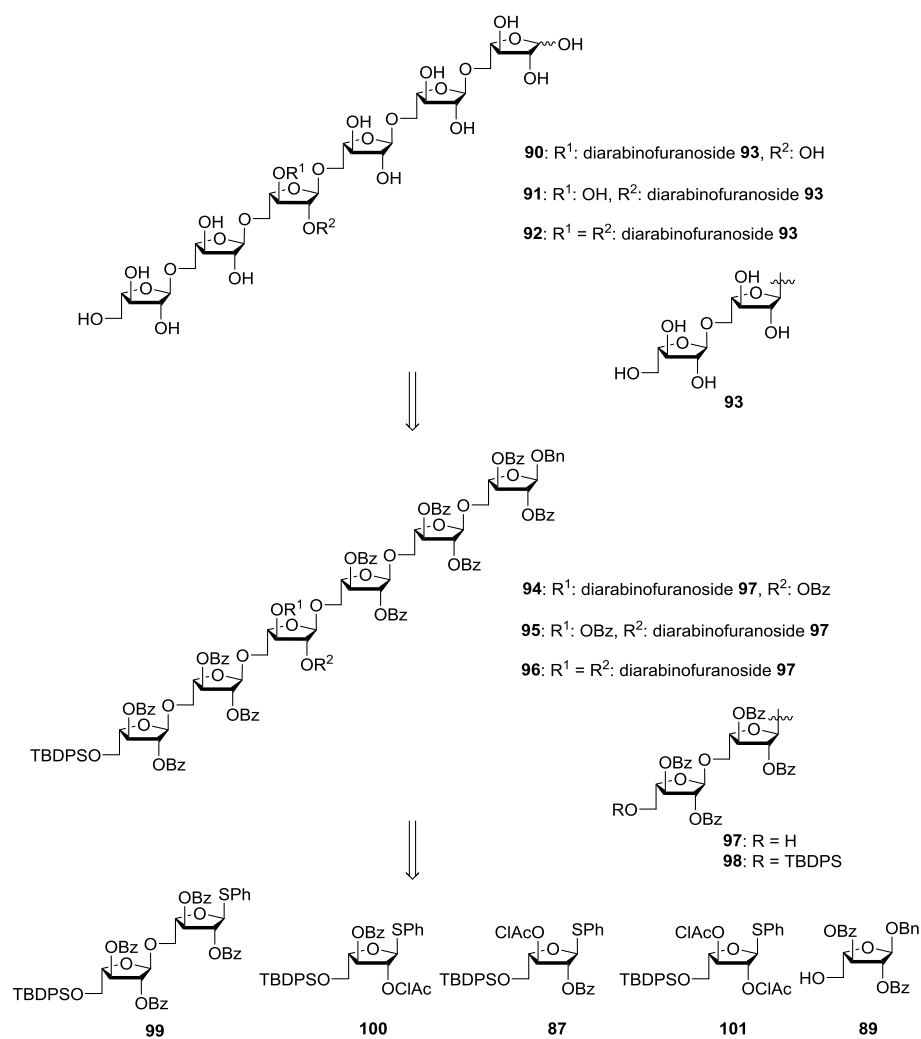
### 3 Results and discussion

The aim of this project was to synthesize branched (1→5) linked  $\alpha$ -L-arabinofuranosides to get a better understanding of the structure of pectin. Three target molecules (**90**, **91**, and **92**, Figure 18) each consisting of a hexasaccharide backbone were chosen as targets. The two octasaccharides **90** and **91** (Figure 18) each carry a disaccharide branching in either the 3- or the 2-position of the fourth monosaccharide from the reducing end, whereas decasaccharide **92** is branched in both the 2- and the 3-position with a disaccharide.

#### 3.1 Retrosynthetic analysis

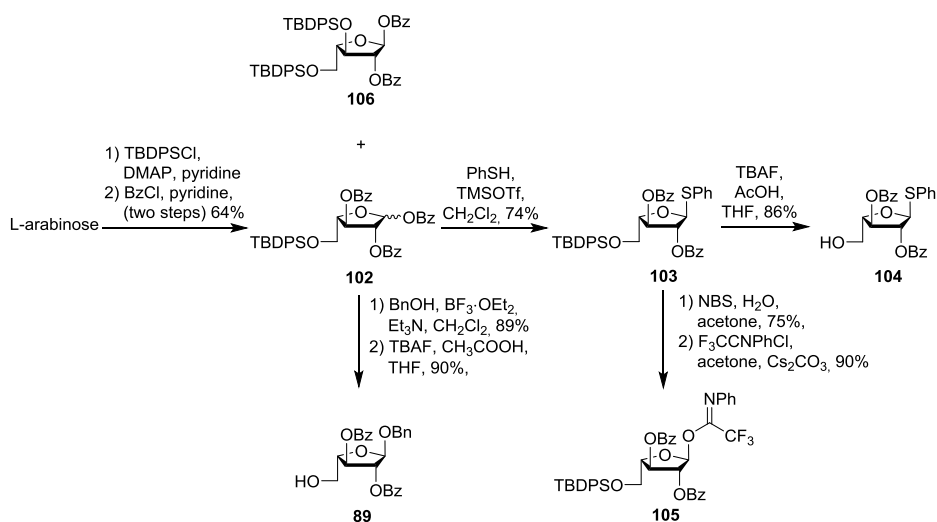
The completely deprotected target molecules **90**, **91** and **92** were envisioned synthesized from the fully protected counterparts **94**, **95**, and **96** (Figure 18). As 2,3-di-*O*-benzoyl- $\alpha$ -L-arabinofuranosyl-(1→5)-2,3-di-*O*-benzoyl- $\alpha$ -L-arabinofuranoside (**98**) recurs several places in the target molecules, a disaccharide donor (e.g. **99**) was an important building block in the synthesis, since it would limit the number of glycosylations. Retrosynthetically speaking, the first disconnection was between the different core hexasaccharides and the branching part. The core hexasaccharide was built up of three different building blocks; a disaccharide donor **99**, a reducing end monosaccharide with a benzyl group on the anomeric position (**89**), and a monosaccharide with an alternative protecting group, to introduce branching. Three differentially protected monosaccharides were needed with an alternative protecting group on either the 2- or the 3-position (**100** and **87**) or on both (**101**). These five building blocks would open for a variety of different oligosaccharide combinations, also the three target molecules **90**, **91** and **92**. The convergent synthetic strategy in which the branching is introduced as the last step will offer other branched oligosaccharide products by slight changes.

Four different protecting groups were used for the building blocks. The benzyl group was used to cap the anomeric position of the growing oligosaccharide, the *tert*-butyldiphenylsilyl (TBDPS) group was used to protect the 5-OH, the chloroacetyl group was used as the alternative protecting group in the monosaccharides, and finally the benzoyl group was used for the remaining hydroxyl groups.



**Figure 18** Building blocks involved in the synthetic strategy of (1→5)- $\alpha$ -L-arabinofuranosides and target molecules **90**, **91** and **92**.

### 3.2 Synthesis of monosaccharide building blocks



**Scheme 8** Synthesis of building blocks.

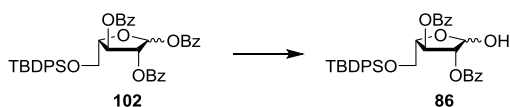
The reducing end monosaccharide **89**, the *N*-phenyl-trifluoroacetimidate donor **105**, and the thioglycoside acceptor **104** were all synthesized from TBDPS protected **102** which was obtained in only two steps from L-arabinose (Scheme 8), inspired by Zhang *et al.*'s work on D-arabinose.<sup>169</sup> The first step took advantage of TBDPS's selectivity for primary hydroxyl groups, then the monosaccharide was perbenzoylated to obtain building block **102**. The TBDPSCl needed to be added slowly to the reaction mixture in order to avoid silylating the 3-position, and thereby obtain 1,2-di-*O*-benzoyl-3,5-di-*O*-*tert*-butyldiphenylsilyl- $\alpha$ -L-arabinofuranoside **106**. Even though it is possible to silylate some secondary hydroxyl groups, no arabinopyranose was observed in the reaction.

To synthesize the reducing end monosaccharide **89** it was first planned to selectively debenzoylate the anomeric position of building block **102** and then introduce the benzyl group. Debenzoylating experiments with  $\text{BF}_3\cdot\text{Et}_2\text{O}$ , dimethylamine in  $\text{H}_2\text{O}$ , hydrazine-acetate in dimethylformamide, and ethane-1,2-diamine, however, did not give good results (Table 3). Following investigations showed that it was possible to benzylate building block **102** directly using  $\text{BF}_3\cdot\text{Et}_2\text{O}$  and benzyl alcohol to achieve the benzyl arabinoside **107**, Table 4. The reaction conditions were adapted from Almendros *et al.*, who selectively benzylated the

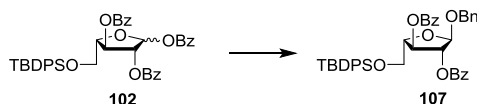


anomeric position of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl- $\alpha$ -L-arabinofuranoside.<sup>170</sup> With 1.2 equivalents of benzyl alcohol the synthesis however only resulted in 26% yield both at 22 °C and 0 °C (Table 4, entry 1 and 2). The major side-reaction was removal of the TBDPS group to obtain reducing end monosaccharide **89**. Triethylsilyl trifluoromethanesulfonate (TESOTf) and TMSOTf were investigated as the Lewis acid (Table 4, entry 3-7) without satisfactory outcome. It was found crucial to use a big proportion (6 equivalents) of benzyl alcohol, as this improved the yields remarkably (Table 4, entry 7-9).  $\text{BF}_3 \cdot \text{Et}_2\text{O}$  was found superior to TMSOTf as Lewis acid and the benzylated arabinofuranoside **107** was obtained in 89% yield from 11 g starting material. The TBDPS group was removed using tetra-*n*-butylammonium fluoride (TBAF) affording reducing end building block **89** in 90% yield (Scheme 8).

**Table 3** Attempted selective debenzoylation of the anomeric position of **102**.



Entry	Conditions	Result	Reference
1	$\text{BF}_3 \cdot \text{OEt}_2$ , $\text{H}_2\text{O}$ , $\text{CH}_2\text{Cl}_2$	All benzoyl groups removed	
2	$\text{Me}_2\text{NH}$ in $\text{H}_2\text{O}$ , pyridine	Many products formed	Nikolaev <i>et al.</i> 1990 <sup>171</sup>
3	DMF, hydrazine-acetate	No selectivity for the anomeric position	Campbell and Tanner 1999 <sup>172</sup>
4	- 0 °C		
5	$\text{H}_2\text{NCH}_2\text{CH}_2\text{NH}_2$ , $\text{CH}_3\text{COOH}$ , THF	No selectivity for the anomeric position	Zhang and Kováč 1999 <sup>173</sup>

**Table 4** Direct benzylation of the anomeric position of **102**, all performed in CH<sub>2</sub>Cl<sub>2</sub>.

Entry	BnOH (equiv.)	Conditions (equiv.)	Temp (°C)	Time (h)	Yield (%)
1	1.2	BF <sub>3</sub> ·OEt <sub>2</sub> (3), Et <sub>3</sub> N (0.8)	22	24	26
2	1.2	BF <sub>3</sub> ·OEt <sub>2</sub> (3), Et <sub>3</sub> N (0.8)	0	24	27
3	1.2	TESOTf (0.05)	0	3	50
4	1.8	BF <sub>3</sub> ·OEt <sub>2</sub> (1.2)	0	1	45
5	1.8	TMSOTf (1)	0	24	36
6	1.8	TESOTf (0.05)	0	24	5 (very little conversion)
7	6	TMSOTf (1.2)	22	3	74
8	6	BF <sub>3</sub> ·OEt <sub>2</sub> (3)	22	24	84
9	6	BF <sub>3</sub> ·OEt <sub>2</sub> (3), 11 g starting material	22	24	89

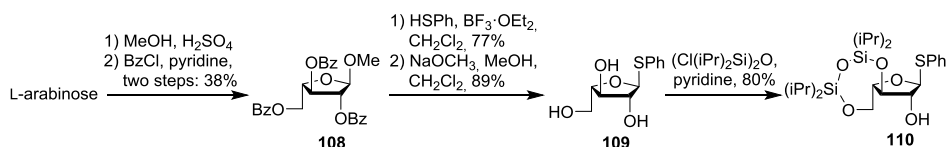
Thioglycoside **103** was obtained from building block **102** in a similar fashion (Scheme 8); thioglycosylation was best mediated by TMSOTf since BF<sub>3</sub>·Et<sub>2</sub>O resulted in poor conversion and many different by-products. Similar poor results using BF<sub>3</sub>·Et<sub>2</sub>O were observed by Wang *et al.*; they achieved better thioglycosylation yields using SnCl<sub>4</sub> as promoter.<sup>174</sup>

*N*-Phenyl-trifluoroacetimidate donor **105** was obtained from thioglycoside **103** by hydrolyzing the anomeric position with *N*-bromosuccinimide (NBS)/water before introducing the acetimidate group by treating the lactol with *N*-phenyl trifluoroacetimidoyl chloride and Cs<sub>2</sub>CO<sub>3</sub> (Scheme 8). Cs<sub>2</sub>CO<sub>3</sub> was found to improve the yield and selectivity for the α product compared to K<sub>2</sub>CO<sub>3</sub>.

Treating thioglycoside **103** with TBAF removed the TBDPS group and afforded acceptor **104** in 86% yield (Figure 8).

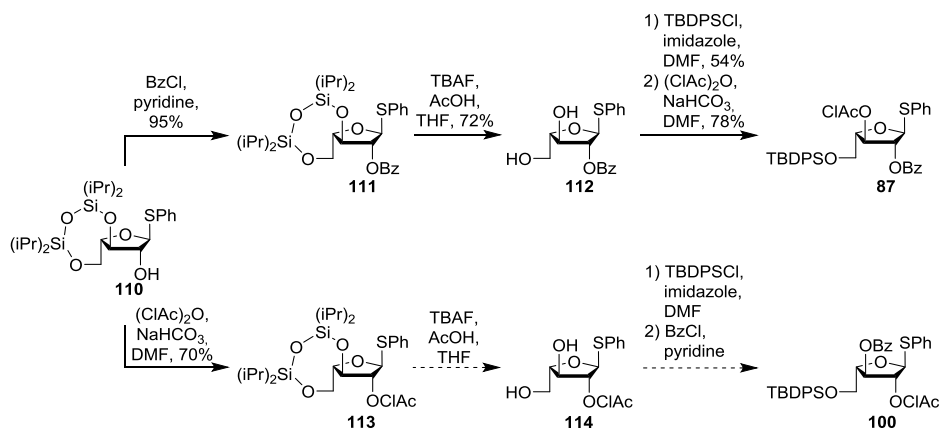
It was envisioned that the two differentially protected monosaccharides **100** and **87** (Scheme 10) could be furnished from the same thioglycoside **110**,<sup>150</sup> which was synthesized in five steps from L-arabinose (Scheme 9). The furanose was produced in an approximate mixture of 1:1 α:β, therefore the maximum yield of

the first two steps was about 50%. The purification was simple, since methyl 2,3,5-tri-*O*-benzoyl- $\alpha$ -L-arabinofuranoside **108** was a crystalline solid, and the  $\beta$ -glycoside was an oil. Then the thiophenyl group was introduced on the anomeric position and the benzoyl groups were removed with the Zemplén conditions before the TIPDS group was introduced to protect the 3- and the 5-position affording compound **110**.



**Scheme 9** Synthesis of TIPDS protected **110**, a precursor for the synthesis of building blocks **100** and **87**.

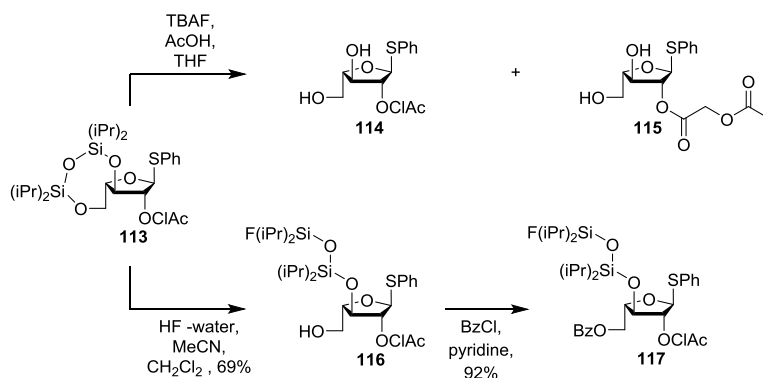
To synthesize monosaccharides **87** and **100** we envisioned protecting the 2-position of compound **110** with either a chloroacetyl or a benzoyl protecting group, followed by TIPDS removal and then TBDPS protecting the 5-position before introducing either a benzoyl or chloroacetyl protecting group on the free 3-position. This strategy gave satisfactory yields for monosaccharide **87** (Scheme 10).



**Scheme 10** Synthesis of monosaccharide **87** and the proposed route to monosaccharide **100**.

However, removing the TIPDS group from compound **113** with a chloroacetyl group in the 2-position resulted in only 45% yield of diol **114**. Diol **115**, where acetic acid had reacted with the chloroacetyl group, was found as a major by-product, see Scheme 11. Gu *et al.* actually reports that TBAF is able to remove the chloroacetyl group completely.<sup>175</sup> Instead it was tested if HF – water could remove the TIPDS group, but this resulted in the TIPDS group still being

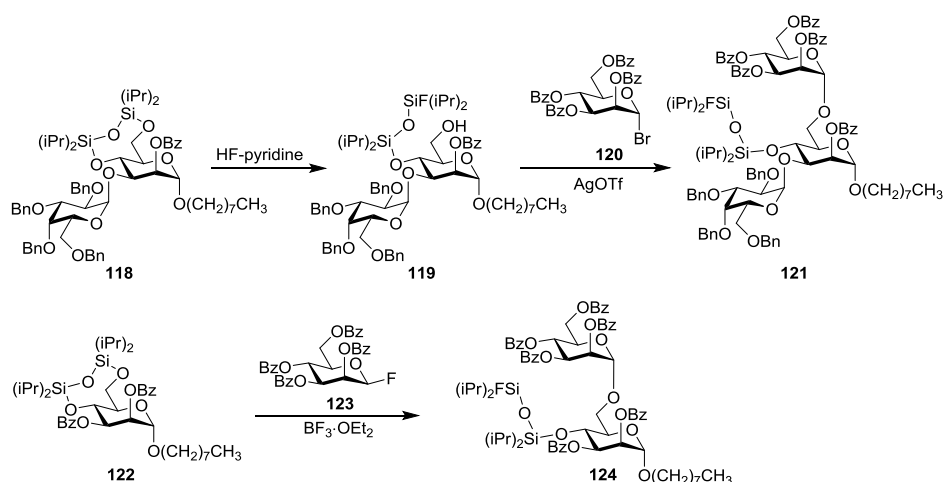
attached to one of the hydroxyl groups. It was hard to determine which position the ring-opened TIPDS group was attached to, so the structure of the product was verified by NMR after reacting the free alcohol with benzoyl chloride, which clearly protected the 5-position to obtain **117** (Scheme 11). Accordingly the product from the ring-opening of the TIPDS group was compound **116**.



**Scheme 11** By-products from deprotection of the TIPDS group of **113**.

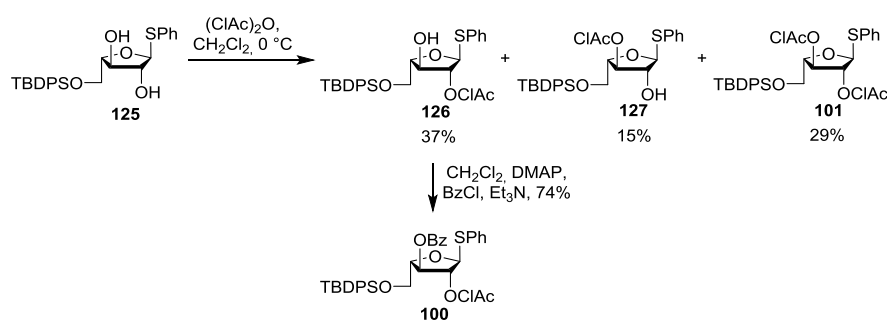
The selective opening of the TIPDS ring was used by Ziegler *et al.* to obtain an acceptor (**119**, Scheme 12) which was used in a glycosylation reaction.<sup>176</sup> It was also found possible to perform a direct glycosylation reaction between a fluoride donor **123** and a TIPDS protected monosaccharide **122** in the presence of BF<sub>3</sub>·OEt<sub>2</sub> to afford (1→6) linked disaccharide **124** (Scheme 12).<sup>177</sup> These conditions were not applicable as an alternative to monosaccharide **87** since we needed an orthogonal protecting group for the 3-position and the remaining TIPDS group was removed with TBAF like the TBDPS group on the 5-position.

Instead monosaccharide **100** was synthesized from the TBDPS protected diol **125** (Scheme 13). It was envisioned that reacting **125** with chloroacetic anhydride could give some selectivity for the 2-position either due to steric or electronic factors, or a combination thereof. The reaction showed a slight selectivity for protecting the 2-position, however, the 3-position was also protected either solely or in combination with the 2-position resulting in **127** and **101**. Since it was possible to separate the two regioisomers **126** and **127** using column chromatography, and since **126** is only one step from monosaccharide **100**, this route was followed despite the low yield. Reacting **126** with benzoyl chloride using 4-dimethylaminopyridine and triethylamine as bases resulted in monosaccharide **100** in 74% yield (Scheme 13). It was found necessary to use milder conditions than standard benzylation conditions using



**Scheme 12** Regioselective opening of the TIPDS ring used by Ziegler *et al.*<sup>176,177</sup>

benzoyl chloride in pyridine, which degraded the starting material. By-product **101** was found useful for synthesizing the third target molecule, the decasaccharide **92** with a hexasaccharide backbone branched in both the 2- and the 3-position of the fourth monosaccharide.



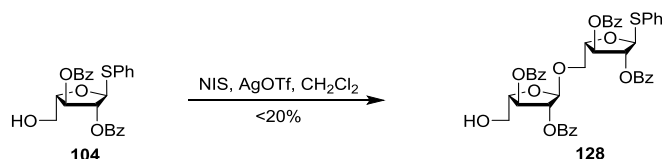
**Scheme 13** Synthesis of monosaccharide **100** in two steps from diol **125**.

### 3.3 Synthesis of disaccharide donor **99**

Initially the possibility of synthesizing disaccharide **128** from only one monosaccharide building block **104** (Table 5) was examined. The glycosylation was promoted by a combination of NIS and either silver triflate or TESOTf and yielded disaccharide **128** in maximum 20% yield (Table 5, entry 1). Experiments showed that it was essential to keep the temperature low and the reaction time short (Table 5, entry 2-3). In all cases the starting material was consumed and thin layer chromatography (TLC) showed production of many by-products, even when the temperature was kept at  $-78^{\circ}\text{C}$ . The same was the case with TESOTf as promoter (Table 5, entry 4). This suggests that

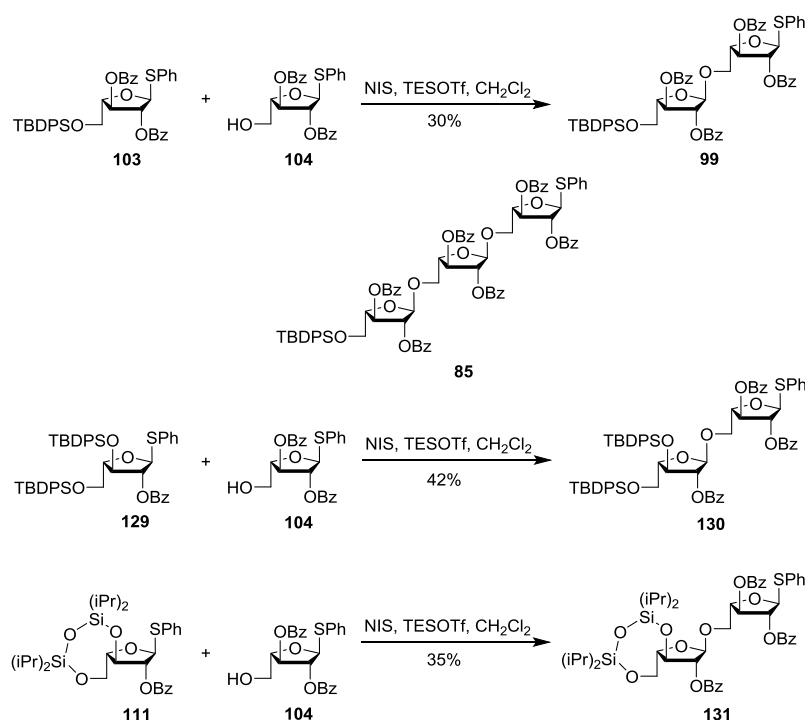
disaccharide **128** is not significantly less reactive than monosaccharide **104**, therefore longer oligosaccharides were formed.

**Table 5** Reaction conditions for the self glycosylation of monosaccharide **104** to synthesize disaccharide **128**.



Entry	Temp	Acid	Time	Yield
1	-78 °C	AgOTf (0.5)	4.5 h	20%
2	-20 °C	AgOTf (0.5)	5 h	2%
3	-78 → 5 °C	AgOTf (0.5)	22 h	<5%
4	-78 °C	TESOTf (0.5)	2 h	1%

The next approach explored the possibility of using thioglycoside **104** as an acceptor in combination with different thioglycoside donors (Scheme 14). The TBDPS protected thioglycoside donor **103** led to the desired disaccharide **99** in 30% yield when 1 equivalent of TESOTf was used (Table 6, entry 1 and 2). The yield was optimized to 35% when catalytic proportions (0.1 equivalents) of TESOTf were used and the donor was added dropwise during the course of the reaction (Table 6, entry 3). The major by-product was the trisaccharide thioglycoside **85**, which either resulted from self-coupling of **104** before reacting with the TBDPS protected thioglycoside **103**, or the desired product **99** reacting once again with acceptor **104**. Either way by-product **85** indicated that the reactivity difference between monosaccharide donor **103** and disaccharide donor **99** was too insignificant to be exploited efficiently.



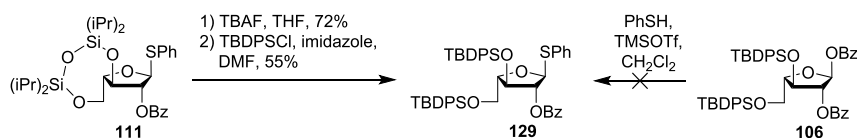
**Scheme 14** Coupling between thioglycoside acceptor **104** and different thioglycoside donors.

**Table 6** Coupling conditions for reaction between **103** and **104**, all performed at -78 °C.

Entry	Conditions (equivalents)	Yield
1	NIS (1), TESOTf (1), 5 hours	30%
2	NIS (1), TESOTf (1), 3.5 hours	30%
3	NIS (1), TESOTf (0.1), acceptor added dropwise over 2 hours, after which the reaction was quenched	35%

Initial experiments with the double TBDPS protected donor **129** showed promising results (Scheme 14), which correlates with Liang *et al.*'s findings for the D-enantiomer, where compound D-**129** is more reactive than the corresponding 3,5-di-*O*-Bn protected counterpart.<sup>178</sup> Thioglycoside **129** was synthesized from tetraisopropylidisiloxane-1,3-diyl (TIPDS) protected **111**, which was desilylated before the 3- and the 5-positions were protected with TBDPS (Scheme 15). It was also investigated whether compound **129** could be synthesized from benzoate

**106**, but neither TMSOTf, boron trifluoride diethyl etherate ( $\text{BF}_3 \cdot \text{Et}_2\text{O}$ ), nor  $\text{SnCl}_4$  as acid were successful (Scheme 15).



**Scheme 15** Synthesis of thioglycoside donor **129**.

Glycosylation between thioglycoside acceptor **104** and TIPDS protected thioglycoside **111** resulted in disaccharide **131** in 35% yield (Scheme 14). This again correlates with observations from Liang *et al.*,<sup>178</sup> who found compound D-**111** to be less reactive than compound D-**129**, but more reactive than the thioglycoside protected with the more strained 3,5-di-*O*-*tert*-butylsilylene ring.

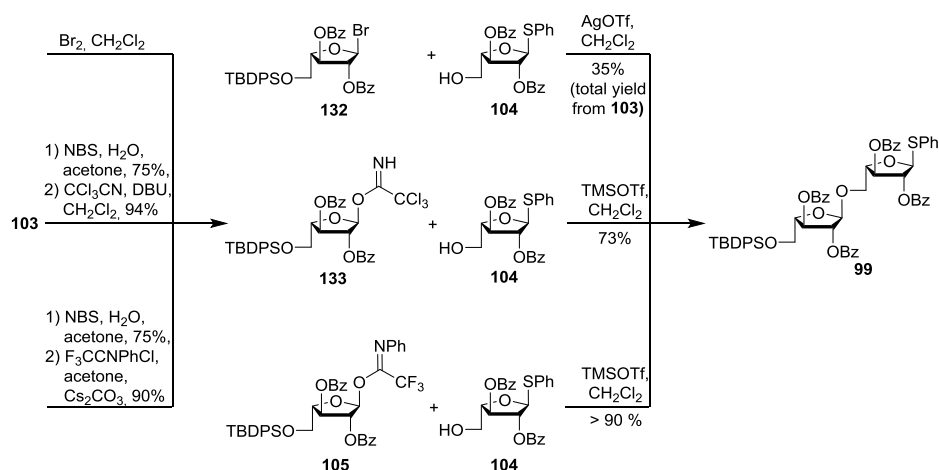
To eliminate the possibility of self-coupling of the thioglycoside acceptor, different glycosylation conditions were examined using three different 5-*O*-TBDPS protected donors. A bromide- (**132**), a trichloroacetimidate- (**133**), and an *N*-phenyl-trifluoroacetimidate (**105**) donor were all prepared from thioglycoside donor **103**. The bromide **132** was prepared directly from the thioglycoside with bromine in  $\text{CH}_2\text{Cl}_2$  and without work-up or characterization of the bromide, it was reacted with acceptor **104** under the Koenigs-Knorr conditions. The synthesis of trichloroacetimidate **133** was similar to the one for the *N*-phenyl-trifluoroacetimidate **105** which was described in section: 3.2 Synthesis of monosaccharide building blocks. First the thioglycoside was hydrolyzed, then the trichloroacetimidate group was introduced using 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) as the base. The trichloroacetimidate donor **133** was not characterized, but used directly in the glycosylation, like the bromide donor **132** to prevent degradation.

Initial results with the three donors suggested that the *N*-phenyl-trifluoroacetimidate donor **105** was superior, yielding disaccharide donor **99** in more than 90% yield (see Scheme 16) as opposed to 73% yield for the trichloroacetimidate **133** and 35% yield (over two steps) for the bromide **132**.

Table 7 offers an overview of the conditions tried for each donor and an overall yield of the glycosylation reactions from the thioglycoside precursor **103**. Silver triflate could also have been used to promote the



glycosylation reaction, since it in some cases have been found to give better yields than TMSOTf.<sup>179</sup>



Scheme 16 Reagents and yields for different donors to give disaccharide donor 99.

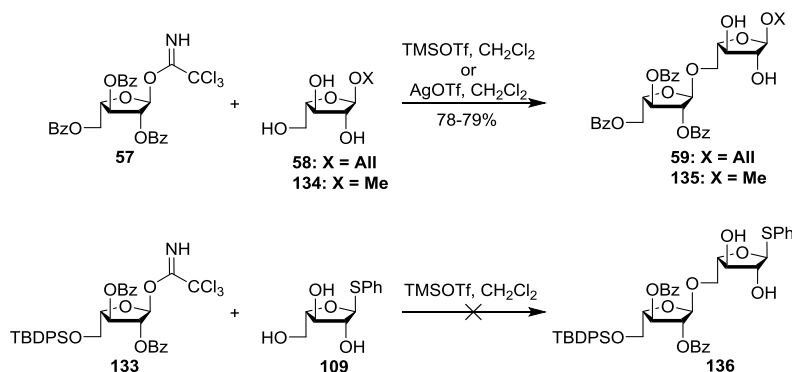
Table 7 Reaction conditions for the reactions in Scheme 16.

Entry	Donor	Conditions (equiv.)	Yield (%) <sup>a</sup>
1		AgOTf, $\text{CH}_2\text{Cl}_2$ , -30 °C, 15 min	23
2	<b>132</b>	AgOTf, $\text{CH}_2\text{Cl}_2$ , -78 °C, 45 min	29
3	<b>132</b>	AgOTf, $\text{CH}_2\text{Cl}_2$ , <i>sym</i> -collidine, -78 °C, 15 min	35
4		TMSOTf (0.4), $\text{CH}_2\text{Cl}_2$ , -30 °C, 1 hour	15
5	<b>133</b>	TMSOTf (0.4), $\text{CH}_2\text{Cl}_2$ , -55 °C, 45 min	55
6		TMSOTf (0.05), $\text{CH}_2\text{Cl}_2$ , -78 °C, 20 min Acetimide prepared using $\text{K}_2\text{CO}_3$	62

7	105	TMSOTf (0.05), CH <sub>2</sub> Cl <sub>2</sub> , -78 °C, 55 20 min Acetimide prepared using Cs <sub>2</sub> CO <sub>3</sub>
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<sup>a</sup>Overall yield from thioglycoside **103**.

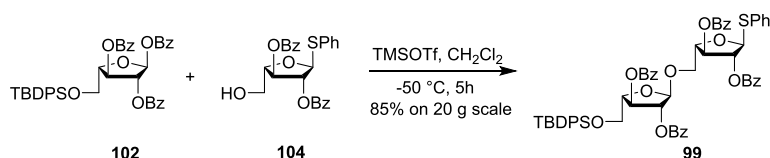
Glycosylation with a fully benzoylated trichloroacetimidate donor **57**, (Scheme 17) was described in the introduction section 1.4.2 (Scheme 5, p. 22).<sup>145,179</sup> Inspired by these results a glycosylation between trichloroacetimidate donor **133** and 1-thio- $\alpha$ -L-arabinofuranoside **109** was examined (Scheme 17). The donor did not show selectivity for the 5-position of the acceptor, and many different products were observed by TLC. No further examinations of these conditions were pursued.



**Scheme 17** Experiments with regioselective glycosylation between trichloroacetimidate **133** and thioglycoside triol **109** encouraged by results from Du *et al.* on glycosylation between trichloroacetimidate **57** and triol **58** or **134** to achieve disaccharide **59** or **135**.<sup>145,179</sup>

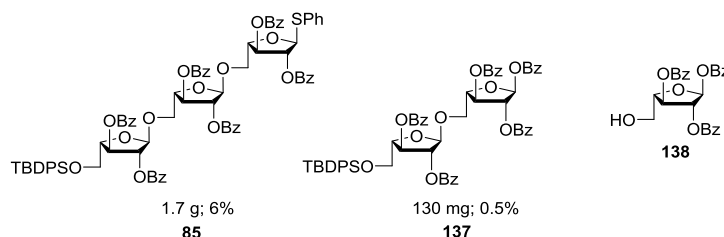
With a good yielding synthetic strategy for the disaccharide donor in hand, the monosaccharide building blocks were scaled up.

Thioglycosylation of **102** on a bigger scale resulted in the formation of a by-product which was identified as disaccharide donor **99**. Although the acidic reaction conditions introduced the thiophenyl group, they also slowly removed the TBDPS protecting group, furnishing thioglycoside acceptor **104**. This encouraged an examination of the donor properties of compound **102** carrying a benzoyl group at the anomeric position. Indeed glycosylation between monosaccharide **102** as a donor and thioglycoside acceptor **104** was possible, yielding disaccharide donor **99** in 85% on a 20 g scale (Scheme 18) in the presence of TMSOTf. Since this strategy involved fewer steps it was more desirable than the one using the *N*-phenyl-trifluoroacetimidate.



**Scheme 18** Synthesis of disaccharide donor **99**.

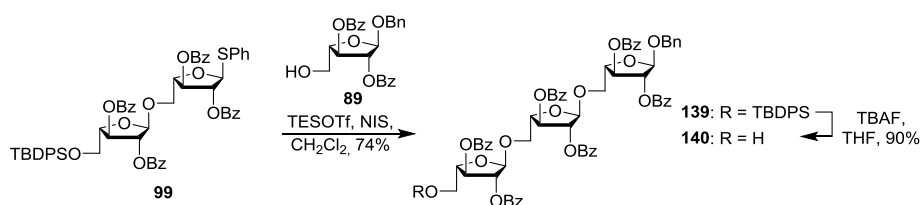
From the large scale synthesis of **99** several by-products (Figure 19) were isolated along with 1.8 g regenerated donor, which corresponded to 1.08 equiv. donor having been used in the reaction. The by-products were identified by NMR as trisaccharide thioglycoside **85** and disaccharide **137** (Figure 19). The presence of these two confirmed that the TBDPS group was slowly removed under the acidic reaction conditions furnishing acceptor **138**, which was not isolated from the reaction. The amount of disaccharide **137** isolated is minute, telling us that the disaccharide is a good donor reacting with acceptor **104** to afford trisaccharide donor **85**.



**Figure 19** By-products in the large scale synthesis of the disaccharide donor (**99**).

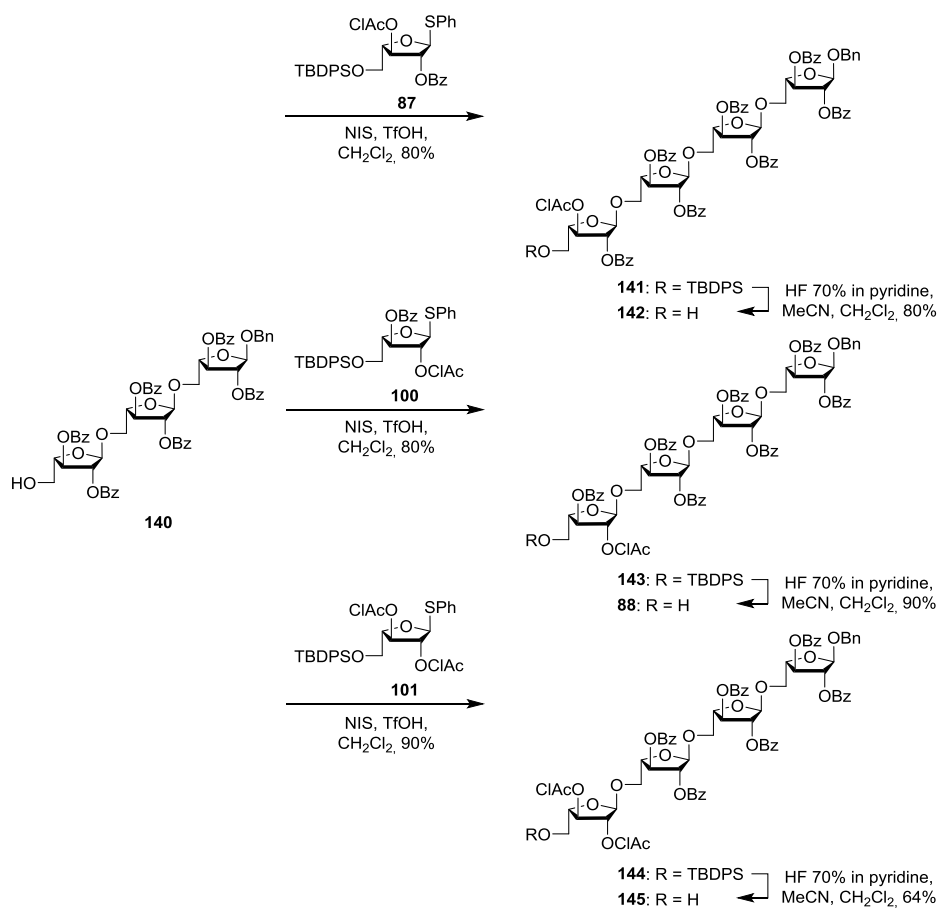
### 3.4 Sequential glycosylations and deprotections to give octa- and decasaccharides

With all the necessary mono- and disaccharide building blocks in hand we next turned our attention to the oligosaccharide synthesis. First the reducing end trisaccharide **139** was synthesized from disaccharide donor **99** and acceptor **89** in 74% yield (Scheme 19). The TBDPS group was removed using TBAF in THF furnishing trisaccharide acceptor **140** in 90% yield.

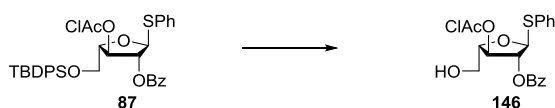


**Scheme 19** Synthesis of reducing end trisaccharide acceptor **140**.

Glycosylation between trisaccharide acceptor **140** and differentially protected monosaccharides **87**, **100** or **101** resulted in tetrasaccharides **141**, **143** and **144** (Scheme 20) in 80-90% yield. The TBDPS group had to be selectively deprotected to enable the next glycosylation reaction. Different conditions were tested on monosaccharide **87** shown in Table 8. As TBAF in THF also reacted with the chloroacetyl group as previously described (Table 8, Entry 1) other conditions were needed. In the presence of two *tert*-butyldimethylsilyl ether (TBS) groups, camphor-10-sulfonic acid (CSA) has been shown to selectively remove the less sterically hindered one.<sup>180</sup> CSA was, however, not able to cleave the TBDPS group from monosaccharide **87** (Table 8, Entry 2), and adding TBAF to the reaction (Table 8, Entry 3), only gave a similar result to Entry 1. HF in water, however, was able to solely cleave the TBDPS group obtaining monosaccharide **146** in 26 - 39% yield (Table 8, Entry 4-6). A long reaction time of 20 h proved essential to the outcome of the reaction (Table 8, Entry 6). HF in pyridine was finally used to cleave off the TBDPS group from the tetrasaccharides to afford the acceptors **142**, **88**, and **145** in 80%, 90%, and 64% respectively (Scheme 20).

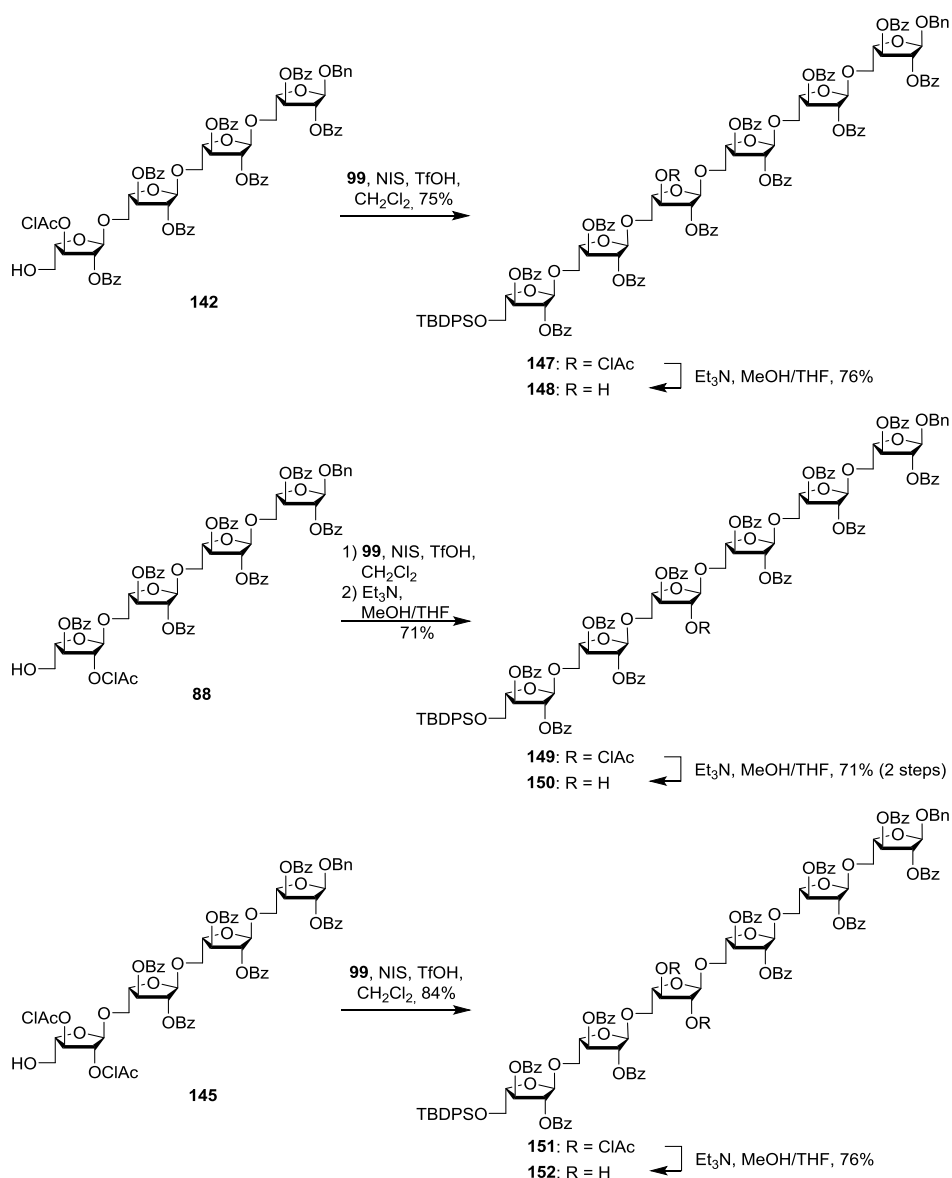


**Scheme 20** Glycosylation- and deprotection conditions for tetrasaccharides **142**, **88**, and **145**.

**Table 8** Examining conditions for removal of TBDPS from a molecule containing a chloroacetyl group.

Entry	Conditions (equiv.)	Yield
1	TBAF, THF	0%, both TBDPS and ClAc hydrolyzed
2	CSA (0.2), 2 h	0%, no conversion
3	TBAF, CSA, MeOH, 3 days	0%, full conversion
4	HF – water (125), MeCN, 43 mM, 4 h	39%
5	HF – water (140), MeCN, CH <sub>2</sub> Cl <sub>2</sub> , 5 mM, 4 h	26%
6	HF – water (140), MeCN, CH <sub>2</sub> Cl <sub>2</sub> , 5 mM, 20 h	70%

Glycosylation between tetrasaccharide **142**, **88**, and **145** and disaccharide donor **99** respectively gave the three core hexasaccharide backbone structures **147**, **149** and **151** (Scheme 21) in 75% and 84% yield for compound **147** and **151**. To reduce the number of purifications hexasaccharide **149** was not isolated and characterized, but directly deprotected to achieve compound **150** in 71% yield over two steps.

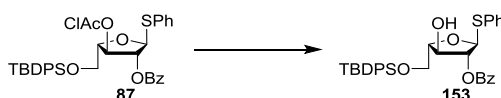


**Scheme 21** Synthesis of the core hexasaccharide acceptors **148**, **150**, and **152**.

To enable branching of the hexasaccharide, the chloroacetyl group needed to be selectively deprotected and test reactions were performed on monosaccharide **87** (Table 9). A variety of the most popular conditions were investigated. The common conditions using thiourea unfortunately only resulted in 25% yield (Table 9, entry 1).<sup>181</sup> Lefeber *et al.*'s conditions (DABCO in ethanol)<sup>182</sup> showed promising results (Table 9, entry 2) on the

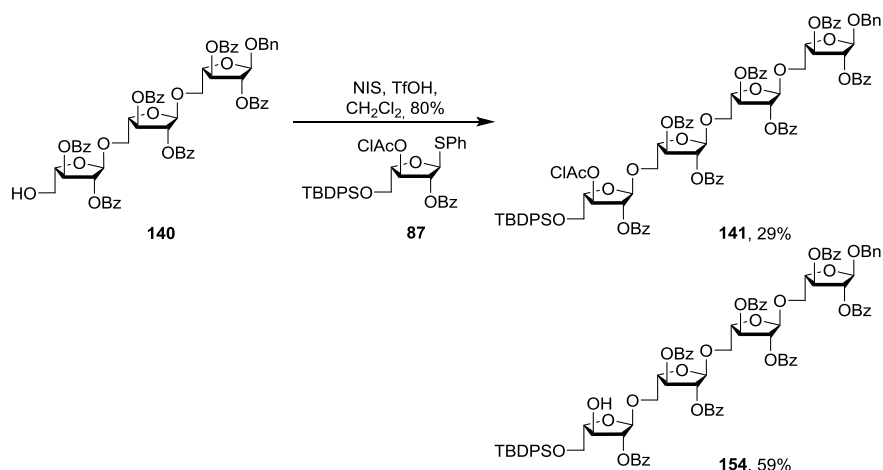
monosaccharide, however the yield dropped when the hexasaccharide was subjected to a variation of the same reaction conditions, in which THF was needed as a co-solvent in order to dissolve the starting material. Instead a dilute solution of NaOMe in THF was tested on monosaccharide **87** (Table 9, entry 3). The chloroacetyl group was removed, but the yield was not better than with DABCO. It was also envisioned that it would be hard to control the unwanted removal of the numerous benzoyl groups on the hexasaccharide. Instead the attention was turned to sodium borohydride as a dechloroacetylating reagent (Table 9, entry 4).<sup>183</sup> The conditions showed promising results, even though the solvent had to be changed from EtOH to a 1:1 mixture of THF-EtOH. Finally triethylamine in MeOH/THF was tested and these conditions resulted in 82% of the monosaccharide alcohol and 76% of the hexasaccharide alcohol. The inspiration to try this came from obtaining unsatisfying glycosylation results for products holding a chloroacetyl group, when the reactions were quenched with triethylamine. In one case the reaction between trisaccharide acceptor **140** and chloroacetyl containing monosaccharide **87** for instance also resulted in tetrasaccharide **154** (Scheme 22), which lacked the chloroacetyl group. After this realization, the glycosylation reaction was poured into a mixture of sat. aq. NaHCO<sub>3</sub> and 10% Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> upon completion of the reaction instead of adding Et<sub>3</sub>N.

**Table 9** Conditions for removal of the chloroacetyl group.



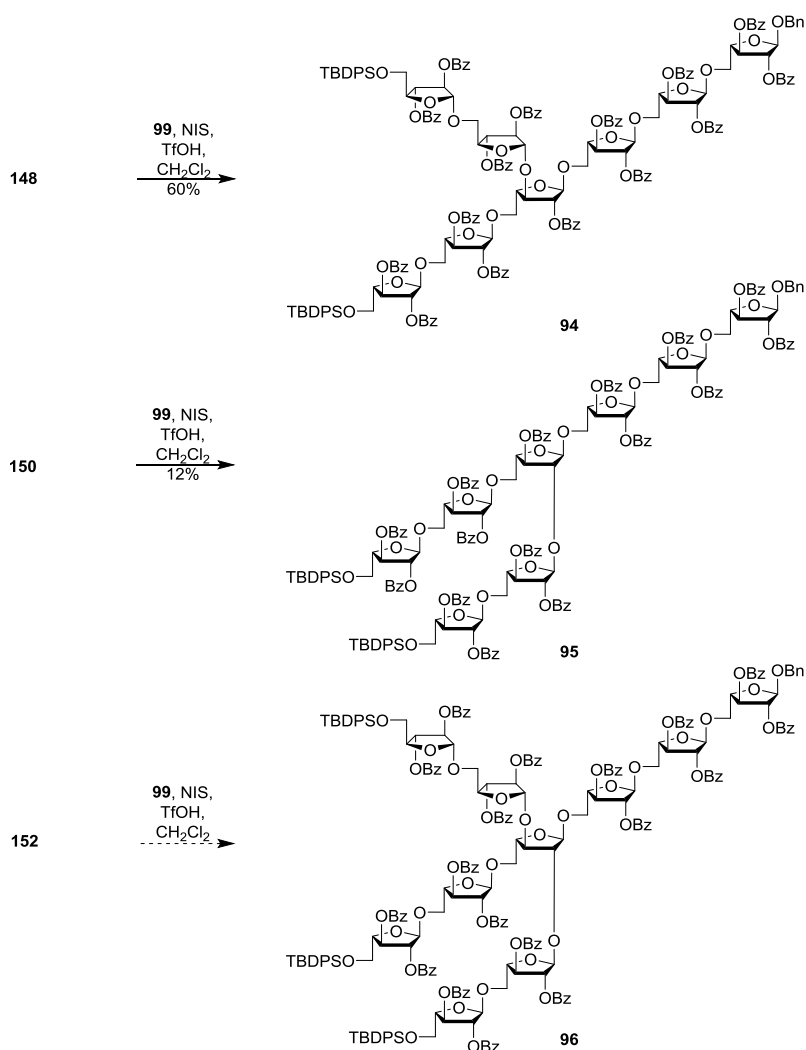
Entry	Conditions	Monosaccharide <b>87</b>	Hexasaccharide <b>147</b>
1	THF, thiourea, Bu <sub>4</sub> NI, NaHCO <sub>3</sub>	25%	
2	DABCO, EtOH/THF 2:1	75%	68%
3	0.02 M NaOMe, THF	71%	
4	NaBH <sub>4</sub> , EtOH/THF 1:1	80%	
5	Et <sub>3</sub> N, MeOH/THF 1:1	82%	76%





**Scheme 22** Side reaction occurring in some glycosylation reaction with products holding a chloroacetyl group.

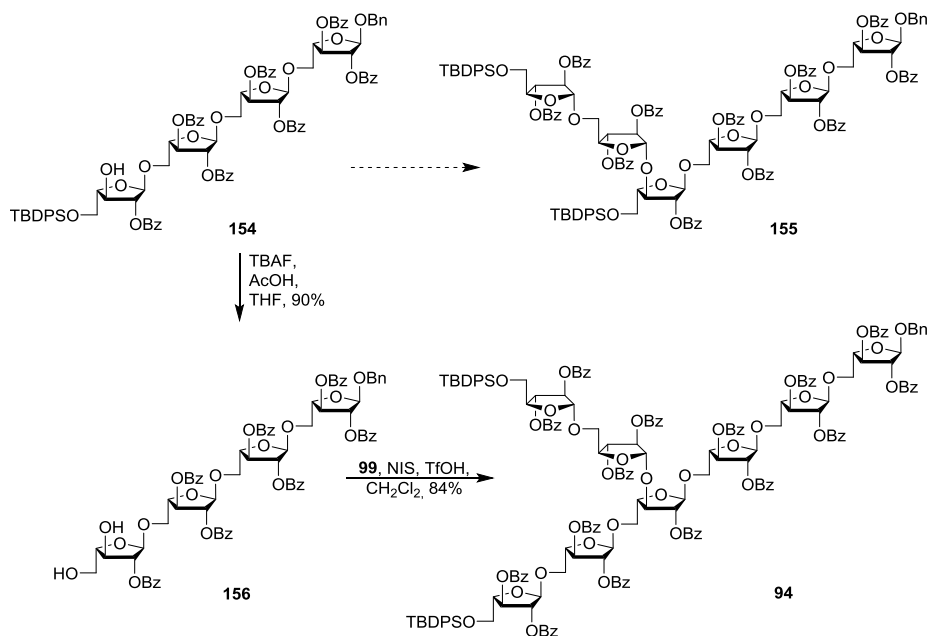
With satisfying conditions for chloroacetyl removal established, the final glycosylation to obtain the two octasaccharides could be performed. The final glycosylation reactions turned out to be cumbersome, as the free 2''' and 3''' hydroxyl groups were more sterically hindered than the primary hydroxyl groups on the other acceptors. However, raising the temperature from around -60 °C to -35 °C and increasing the reaction time made it possible to obtain octasaccharide **94** in 60% yield. Only one attempt of synthesizing regioisomeric hexasaccharide **95** was performed, and that resulted in an unacceptable low yield of 12% (Scheme 23). Compound **95** was only characterized by NMR. Knowing the  $R_f$  value of compound **95** and meanwhile having collected more knowledge about glycosylation of **94** hopefully will help improve the yield. The glycosylation between diol **152** and disaccharide **99** to afford decasaccharide **96** was not examined yet, due to time limitations.



**Scheme 23** Glycosylation reactions to obtain fully protected target molecules **94**, **95** and **96**.

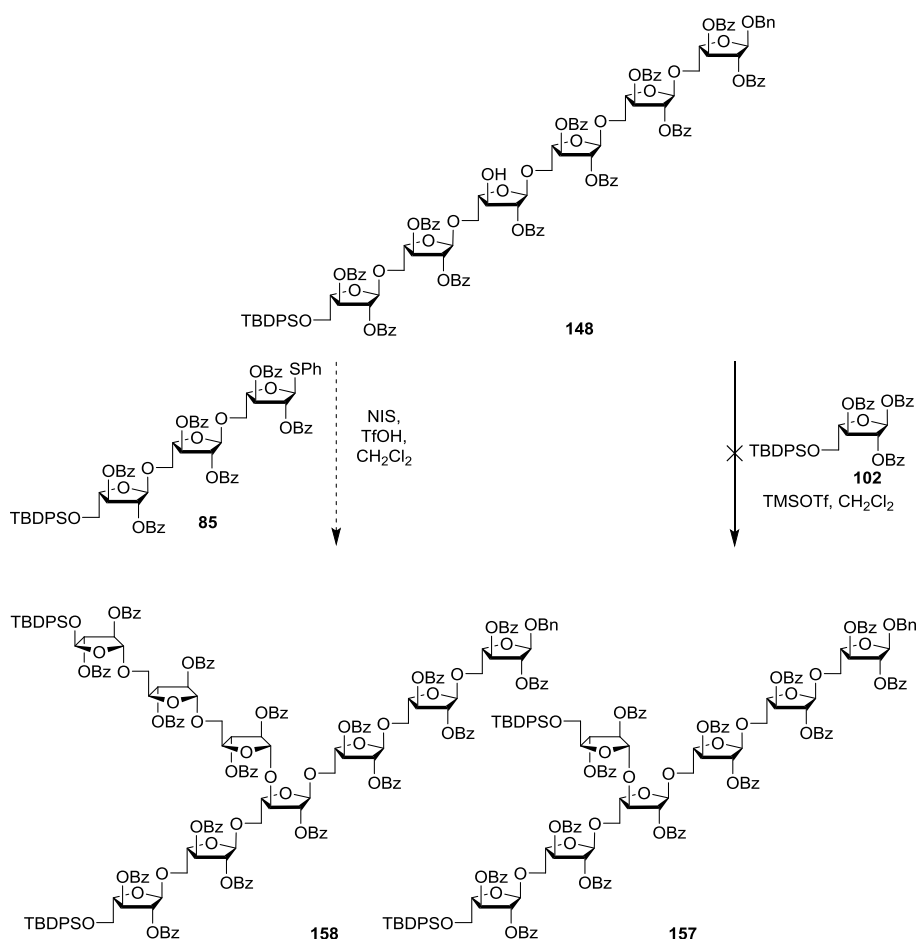
To gain more knowledge about the sterically hindered 3-position, glycosylation between disaccharide donor **99** and tetrasaccharide **154** with a free hydroxyl group in the 3-position of the fourth monosaccharide to synthesize hexasaccharide **155** was considered (Scheme 24). However, it would not be possible to obtain target octasaccharide **94** from the branched hexasaccharide **155**, since both reducing ends of the molecules would be capped with a TBDPS group. If it would be possible to remove one TBDPS group selectively, it would most likely be the one at the branching point. This would, however, only make it possible to extend either the branching or both the backbone and the branching at the same time.

Another possibility would have been to introduce a different disaccharide donor without a TBDPS group on the 5'''-position, but that was not attempted. Instead the TBDPS group was cleaved from tetrasaccharide **154** using TBAF in THF, as there at this point was no need to worry about the chloroacetyl group. This afforded diol **156** in 90% yield (Scheme 24). Then a double glycosylation was performed with disaccharide donor **99** to obtain octasaccharide **94** in 84% yield. As for the glycosylation between the hexasaccharide backbone **148** and disaccharide donor **99**, the reaction temperature needed to be raised to  $-35\text{ }^{\circ}\text{C}$  to obtain full conversion.



**Scheme 24** Possibilities of examining the acceptor properties of the sterically hindered 2'''-position.

To show the advantage of the synthetic strategy, the synthesis of another two oligosaccharides were planned: Nonasaccharide **158** and heptasaccharide **157** (Scheme 25). The reaction between hexasaccharide **148** and monosaccharide donor **102** used to synthesize disaccharide donor **99** was undertaken, without obtaining any heptasaccharide. The more acidic conditions probably destroyed the hexasaccharide before the oligosaccharide was synthesized. The equivalent thioglycoside donor (**103**) is expected to give better results, but the glycosylation was not undertaken.



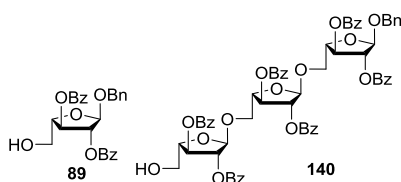
**Scheme 25** Possible synthesis of two oligosaccharides with different branchings in the 3'''-position.

### 3.5 Global deprotection

To reach the target molecules **90** and **91**, it was necessary to deprotect the fully protected octasaccharides **94** and **95**. It was envisioned to remove the protecting groups in the following order: 5-*O*-TBDPS groups, the anomeric benzyl group and last the benzoyl groups. That way the compounds would be kept as apolar as possible, in order to facilitate handling, as oligosaccharides only holding very few protecting groups are insoluble in most organic solvents. Experience in the group also suggested that purification by chromatography on silica (also considering reverse phase chromatography) was remarkably more challenging with very polar compounds.

By testing conditions on monosaccharide **89** and trisaccharide **140** (Table 10), the anomeric benzyl group turned out to be difficult to remove. Palladium on carbon in H<sub>2</sub> atmosphere in different solvents and under different temperatures either led to no conversion or decomposition of the starting material at higher temperatures. Methanol was used as solvent with THF as co-solvent to dissolve the starting material in entry 1 with **89** as starting material. Following, it was found more advantageous to use ethanol as solvent with as little EtOAc as possible to dissolve the oligosaccharide (Table 10, Entry 2-6, 8-10). One experiment was also conducted in which water/THF was used as solvent system (Table 10, Entry 7). It was attempted to add formic acid in combination with the H<sub>2</sub> atmosphere (Table 10, Entry 2-5, 8-10), with no significant improvement. The reactions were performed at temperatures ranging from 20 °C to 90 °C. At temperatures below 60 °C no reaction was observed and at 60 °C a small development was seen, however, the reaction did not come to completion even after seven days. Raising the temperature to 90 °C slowly decomposed the starting material. Instead, palladium hydroxide on carbon in ethanol and ethyl acetate under acidic conditions was tried and this exclusively removed the benzyl group at 39 °C from trisaccharide **140** over 7 days (Table 10, Entry 11).

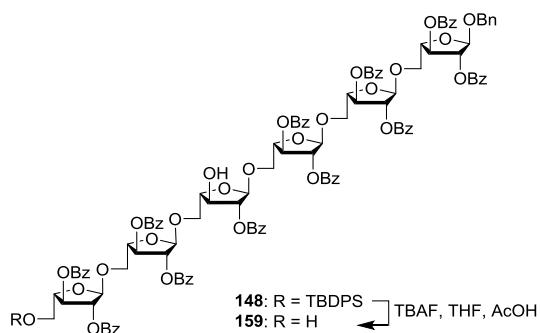
**Table 10 Overview of attempted conditions for removal of the anomeric benzyl group from hexasaccharides.**



Entry	Starting material	Conditions	Outcome
1	<b>89</b>	Pd/C, H <sub>2</sub> , MeOH, THF, 20 °C	No reaction
2	<b>89</b>	Pd/C, H <sub>2</sub> , EtOH, EtOAc, CHOOH, 20 °C	No reaction
3	<b>89</b>	- 38 °C	No reaction
4	<b>89</b>	- 60 °C	<20% yield
5	<b>89</b>	- 90 °C	Starting material decomposed
6	<b>140</b>	Pd/C, H <sub>2</sub> , EtOH, EtOAc, 20 °C	No reaction
7	<b>140</b>	Pd/C, H <sub>2</sub> , H <sub>2</sub> O, THF	No reaction
8	<b>140</b>	Pd/C, H <sub>2</sub> , EtOH, EtOAc,	No reaction

9	<b>140</b>	CHOOH, 20 °C - 60 °C	Very little happened
10	<b>140</b>	- 90 °C	Starting material decomposed
11	<b>140</b>	Pd(OH) <sub>2</sub> -C, H <sub>2</sub> , EtOH, EtOAc, CHOOH	Full conversion
12	<b>159</b>	Pd(OH) <sub>2</sub> -C, H <sub>2</sub> , EtOH, EtOAc, CHOOH	Full conversion

Without the complication from the chloroacetyl group, the TBDPS group was removed from hexasaccharide **148** using TBAF in THF (Scheme 26). The reaction was followed on TLC and diol **159** was confirmed by LCMS. Afterwards the benzyl group was removed (Table 10, Entry 12). The product was confirmed by LC-MS.



**Scheme 26 Selective removal of the TBDPS groups from hexasaccharide 148.**

Oligosaccharides with a free anomeric hydroxyl group develop a brown spot on TLC when heated with a heat gun. This makes it easier to follow the reaction, as the bigger oligosaccharides are not expected to change  $R_f$  value upon deprotection of just one benzyl group. For this reason it is also crucial to obtain full conversion, as it would be difficult to separate them on column chromatography.

Finally the benzoyl groups were to be removed using the Zemplén conditions to reach the target molecules **90** and **91**, however, these reaction have not yet been performed.

### **3.6 Conclusion**

A route to three hexasaccharide backbone structures was completed. Two of the hexasaccharides were branched with a diarabinofuranoside to accomplish the first reported synthesis of two fully protected octasaccharides.

## 4 Oligosaccharide linker system and transglycosylation assay

The work outlined in the following chapter was conducted at the University of Copenhagen in the group of Professor William G. T. Willats during a five month external research stay. The work was primarily divided into two parts, which are described below: Implementation of a linker system for oligosaccharides and development of a transglycosylation assay.

### 4.1 Linker system for oligosaccharides

#### 4.1.1 Background

Oligosaccharide microarrays are powerful tools for high throughput analysis, as many different molecular interactions can be evaluated simultaneously using only small amounts of analytes.<sup>184</sup> An essential part of the technique is being able to immobilize the carbohydrate molecules to an array surface. Bovine serum albumin (BSA) is a protein that can conjugate to oligosaccharides through reductive amination. BSA can then bind to a surface, e.g. nitrocellulose, via non-covalent interactions.<sup>184,185</sup> BSA is often chosen as a carrier molecule because BSA-based neoglycoproteins are multifunctional resources that can be used, not just for microarray production, but also as immunogens, and as components of other assays for which immobilization is required.<sup>8</sup> The two main difficulties related to BSA conjugation are 1) the bulky nature of BSA, which can lead to difficulty for enzymes acting on the bound oligosaccharides and 2) the oligosaccharides are often conjugated to BSA through reductive amination with sodium cyanoborohydride, which results in a ring-opened monosaccharide between the oligosaccharide and the protein (Figure 20).<sup>8</sup> These modifications are likely to interfere with the activity of reducing end-acting probes or enzymes. Another disadvantage connected to ring-opening of the reducing end monosaccharide is loss of information, since e.g. a pentasaccharide afterwards is only recognized as a tetrasaccharide. As discussed in the rest of the thesis, oligosaccharide synthesis can be difficult and time-consuming, therefore destroying one monosaccharide is rather wasteful.

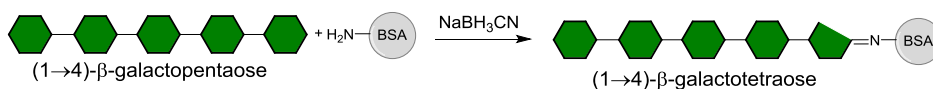
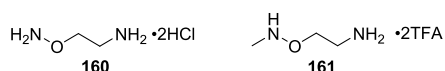


Figure 20 Adapted and modified from Pedersen *et al.*<sup>8</sup>



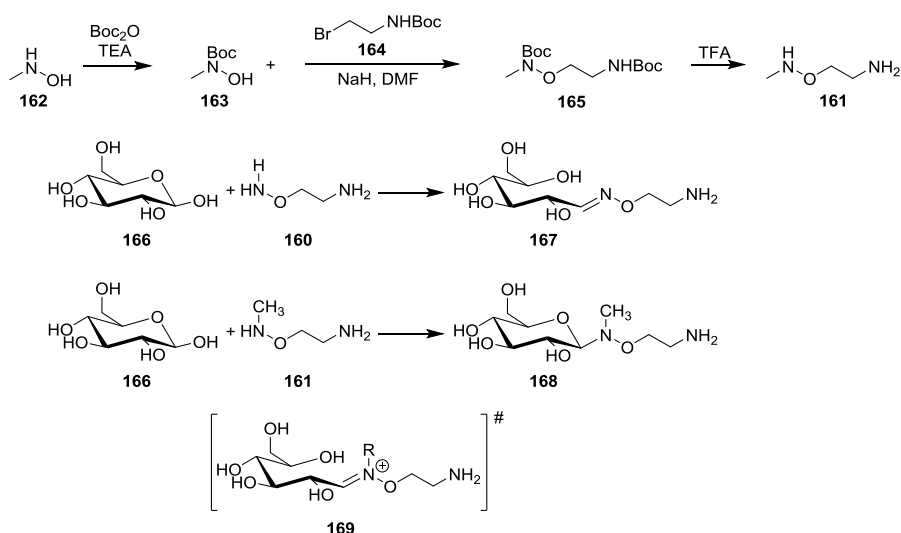
#### 4.1.2 Development of the linker system

Two small compounds were investigated as alternative linkers to BSA: a commercially available small compound, 2-aminoethoxyamine dihydrochloride (**160**, Figure 21), and a similar synthetic linker, (2-aminoethoxy)methylamine ditrifluoroacetic acid (**161**, Figure 21).<sup>186</sup> Compound **161** was synthesized from *N*-methylhydroxylamine (**162**, Scheme 27), which was protected with a Boc group before it was reacted with *N*-Boc-protected 2-aminoethyl bromide (**164**) to afford compound **165**. The Boc groups were removed using trifluoroacetic acid (TFA) to obtain linker **161**. The dihydrochloride of **161** is now also commercially available.



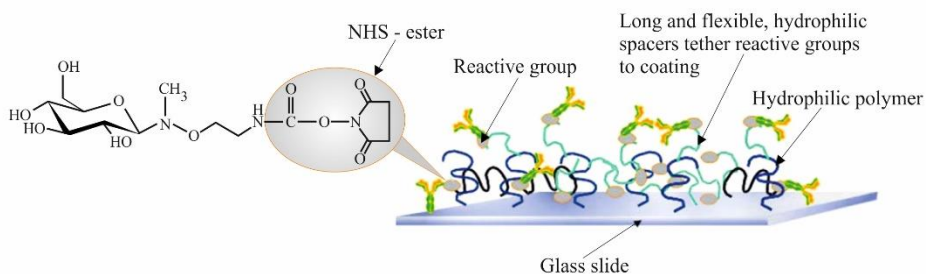
**Figure 21 Chemical structures of the two small linkers used.**

Scheme 27 also illustrates the conjugation between a carbohydrate, in this case represented by glucose (**166**), and the two different linkers **160** and **161**. The conjugation between linker **160** and a carbohydrate has been found mainly to produce the open-ring oxime form of the reducing end monosaccharide, as shown for compound **167**. Linker **161** on the other hand causes a high degree of the ring-closed form of the reducing end monosaccharide like shown for compound **168**.<sup>187,188</sup> The conjugation reactions both go through an oxyiminium intermediate (**169**) which, for R = H, can stay on the open-ring form by deprotonation, however, when R ≠ H, a ring closing-reaction takes place to obtain a neutral product. The last case offers a possibility of preservation of information for the entire conjugated oligosaccharide.



**Scheme 27** Synthesis of the commercial linker 161 and illustration of how the two linkers 160 and 161 react with a carbohydrate.

Six different oligosaccharides (arabinohexaose (Ara6), arabinooctaose (Ara8), mannotetraose (Man4), mannohexaose (Man6), xylotetraose (Xyl4), and xylohexaose (Xyl6)), were each conjugated to the two different linkers in aqueous acetate buffer at 37 °C for two days. Control experiments, in which the oligosaccharides were conjugated to BSA, were also performed. Slide H has a three-dimensional thin film coating, which is chemically functionalized with long polyethylene glycol chains terminated with *N*-hydroxysuccinimide esters, which very easily react with amines (Figure 22). Some of each of the Xyl6 and Ara8 linked sugars were also purified by elution through a carbograph column and spotted on the array.



**Figure 22** Illustration of the Nexterion® slide H coating.<sup>189</sup>

After printing, the glass slides were incubated with blocking buffer, which is used to block the part of the slide not being exposed to samples, to prevent nonspecific binding of the antibodies to the array. Each array was printed 16 times side by side on the same glass slide, which made it possible to test identical arrays against many different monoclonal antibodies simultaneously.

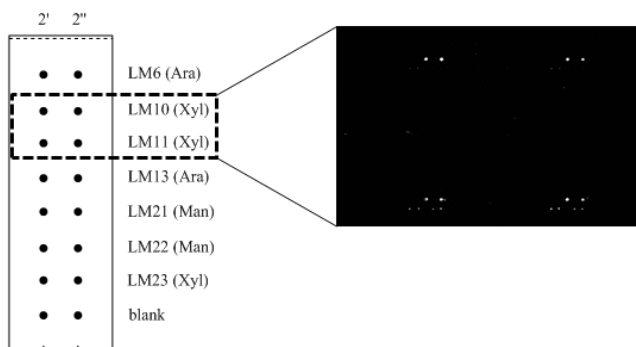
Monoclonal antibodies (mAb) are immensely valuable molecular probes for carbohydrate research.<sup>8</sup> They are monospecific antibodies made by identical immune cells and they serve to detect or purify a given substance by specifically binding to it. Primary antibodies bind directly to antigens or proteins whereas secondary antibodies bind to another (primary) antibody. The secondary antibody can carry a fluorescent tag for detection. mAbs with specificity for plant cell-wall components have been developed by several research groups. The ones used in this set of experiments are listed in Table 11 with an overview of their specificity and origin. The secondary mAb should correspond to the origin of the primary mAb.

**Table 11 An overview of the specificity and origin of the primary antibodies used in this study.**

<b>Code</b>	<b>Specificity</b>	<b>Origin</b>
LM6	(1→5)- $\alpha$ -L-arabinan	Rat <sup>190</sup>
LM10	(1→4)- $\beta$ -D-xylan	Rat <sup>191</sup>
LM11	(1→4)- $\beta$ -D-xylan/arabinoxylan	Rat <sup>191</sup>
LM13	AGP	Rat <sup>192</sup>
LM15	Xyloglucan (XXXG motif)	Rat <sup>193</sup>
LM21	(1→4)- $\beta$ -D-mannan/galactomannan /glucomannan	Rat <sup>194</sup>
LM22	(1→4)- $\beta$ -D-mannan/galactomannan/ glucomannan	Rat <sup>194</sup>
LM23	(1→4)- $\beta$ -D-xylan	Rat <sup>8</sup>
LM24	Xyloglucan	Rat <sup>8</sup>
BS-400-2	(1→3)- $\beta$ -D-glucan	Mouse <sup>195</sup>
BS-400-4	(1→4)- $\beta$ -D-mannan	Mouse <sup>196</sup>

Figure 23 offers an overview of which arrays were treated with which antibodies. The primary antibodies are listed to the right of the slide and the two columns are probed with two different batches of the same secondary antibody indicated on the top of the slide. LM10 and LM11 were the antibodies which

gave the best response, and therefore a cut of this section of the scanned slide is shown to the right of the illustrated glass slide for LM10 and LM11 as indicated on Figure 23. The scanned slide clearly showed the BSA-conjugated sugars (the bigger white spots). The purified new linker-conjugated sugars also showed, however, the signals were not as extensive (the smaller white spots underneath the bigger white spots). The non-purified linker-sugars were not detected.



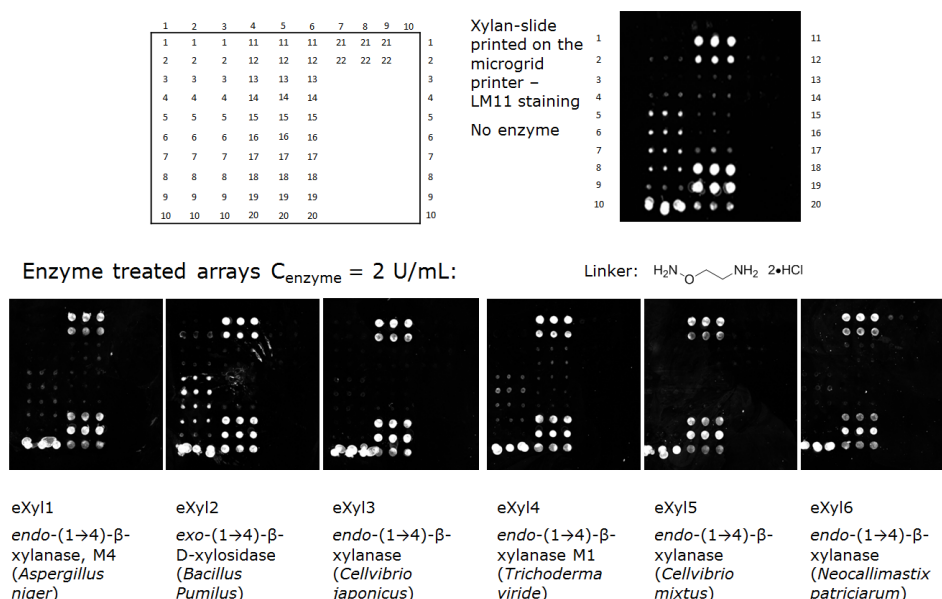
**Figure 23** Illustration of a glass slide indicating which array has been probed with which primary (vertical) and secondary (horizontal) antibodies, and a cut of the scanned slide in which the BSA conjugated xylans and the purified linker-xylan conjugates show.

Xyl3 and Xyl6, conjugated with linker **160** without purification, were spotted on a new glass slide using the contact microgrid printer in concentrations of 1 mM and 0.25 mM. The same two sugars, conjugated with the same linker, were purified and spotted on the glass slide in concentrations of 4 mM, 1 mM, 0.25 mM, and 0.0625 mM, in combinations with the purified samples which were reduced by sodium cyanoborohydride before it was spotted in a concentration of 2 mM. Finally both sugars conjugated to BSA through reductive amination were spotted in concentrations of 1 mg/mL, 0.25 mg/mL, and 0.0625 mg/mL, and both non-conjugated sugars were printed as control experiments. The grid in the upper left corner of Figure 24 illustrates which sample was spotted in which position of the scanned arrays (which are all identical). The legend below Figure 24 explains the numbers in the grid.

All the arrays in Figure 24 were stained with LM11, as this was the antibody producing the best results in the previous experiment. All the samples in the upper array produced visible spots, except for the two sugars printed without a linker (sample 21 and 22) and the two unpurified samples in the lowest concentration (0.25 mM) (sample 1 and 3).

The printer used to print the arrays was found essential, as much smaller concentrations of conjugated sugar were visible when printing on the contact microgrid printer (62  $\mu$ M) versus the piezoelectric non-contact arrayjet printer (22 mM).

The resulting six arrays in Figure 24 were exposed to different enzymes before probing with the antibodies. The spots originating from sugar conjugated to linker **160** (sample 1-9 and 13-17) were no longer as prominent or not even present after treatment with enzymes. This proves that the enzymes were able to react with the sugars conjugated to the new linker but not with the sugars conjugated to BSA. The *exo*-acting hydrolase eXyl2 did not perform as well as the *endo*-acting hydrolases. The immobilization of the oligosaccharides onto the surface probably caused steric hinderence between site of action and the enzyme.



**Figure 24** Arrays printed on the microgrid printer. The spreadsheet, of which the legend is given below (A: linker 160, B: BSA), show the position of each sample on the arrays. The upper array was not treated with any enzyme, while the lower six arrays were treated with six different enzymes before incubated with the mAb, LM11.

- |                                  |                                  |
|----------------------------------|----------------------------------|
| 1 Xyl6A, unpurified, C = 0.25 mM | 5 Xyl6A, purified, C = 4 mM      |
| 2 Xyl6A, unpurified, C = 1 mM    | 6 Xyl6A, purified, C = 1 mM      |
| 3 Xyl3A, unpurified, C = 0.25 mM | 7 Xyl6A, purified, C = 0.25 mM   |
| 4 Xyl3A, unpurified, C = 1 mM    | 8 Xyl6A, purified, C = 0.0625 mM |

9 Xyl6A, purified, aminated, C = 2 mM	16 Xyl3A, purified, C = 0.0625 mM
10 Xyl6B, 1 mg/mL	17 Xyl3A, purified, aminated, C = 2 mM
11 Xyl6B, 0.25 mg/mL	18 Xyl3B, 1 mg/mL
12 Xyl6B, 0.0625 mg/mL	19 Xyl3B, 0.25 mg/mL
13 Xyl3A, purified, C = 4 mM	20 Xyl3B, 0.0625 mg/mL
14 Xyl3A, purified, C = 1 mM	21 Xyl3 not conjugated
15 Xyl3A, purified, C = 0.25 mM	22 Xyl6, not conjugated

### 4.1.3 Conclusion

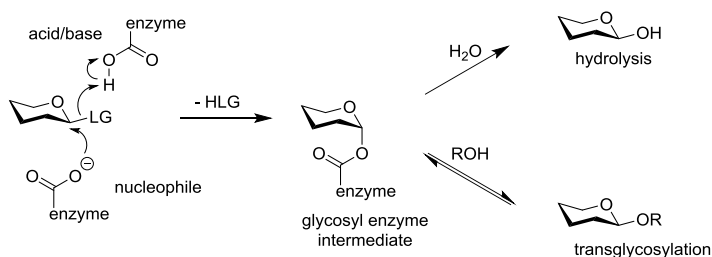
The two small linkers **160** and **161** were found applicable as linkers for oligosaccharides. They showed promising results for enzyme activity, as reactions were feasible between different enzymes and the linked oligosaccharides, opposed to the hindered reactions between the same enzymes and BSA conjugated sugars.

Further studies in the group found that it was possible to print concentrations down to 200  $\mu$ M on the array jet printer by adding 20% peg400 to the samples. Other studies suggested that both linkers had similar efficiency in relation to enzyme activity. It was, however, decided primarily to continue the work with linker **161** because of its ability to maintain the closed form of the reducing end sugar.

## 4.2 Transglycosylation assay

There is an increasing interest to identify the different enzymes involved in the biosynthesis of oligosaccharides. In this project a new method to detect xyloglucan endotransglycosylase (XET)<sup>197</sup> activity using a fluorescently labeled xyloglucan (XGO\*) was developed. The XET was delivered from Novozymes A/S.

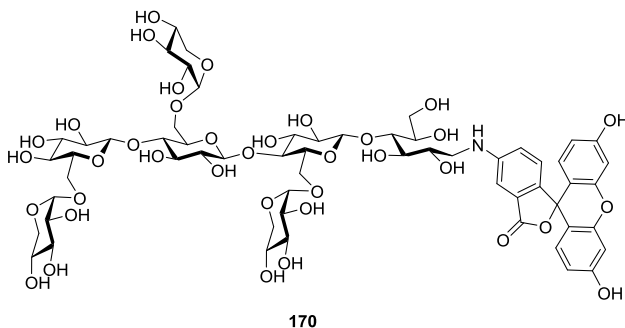
Transglycosylases are a class of glycosyl hydrolase (GH) enzymes that catalyze a glycosylation reaction, e.g. between an oligosaccharide and a polysaccharide (Figure 25).<sup>198</sup> This is the case for XET, which catalyzes the formation of xyloglycans. Only very few transglycosylases are known, of which XET is the one most extensively studied.<sup>199</sup>



**Figure 25 Mechanism for the transglycosylase activity.**

It was believed that the known XET with GH activity could form the basis of a high-throughput array for identification of transglycosylase activity in plant tissue using a fluorescently labeled oligosaccharide. Since readily available nitrocellulose based arrays only retain larger oligosaccharides DP ~15-20, a small oligosaccharide with a fluorescent tag should not stick to nitrocellulose. After transglycosylation with a polysaccharide it would, however, be possible to detect binding of the fluorescent polysaccharide on nitrocellulose.

Beatrice Bonora from Professor Clausen's group synthesized a xyloglucan heptasaccharide (XGO) fluorescently labeled with 5-aminofluorescein to obtain compound **170** (Figure 26), hereafter referred to as XGO\*. The binding affinity of this fluorescent oligomer was tested towards nitrocellulose and as anticipated, the XGO\* was washed away with PBS buffer.



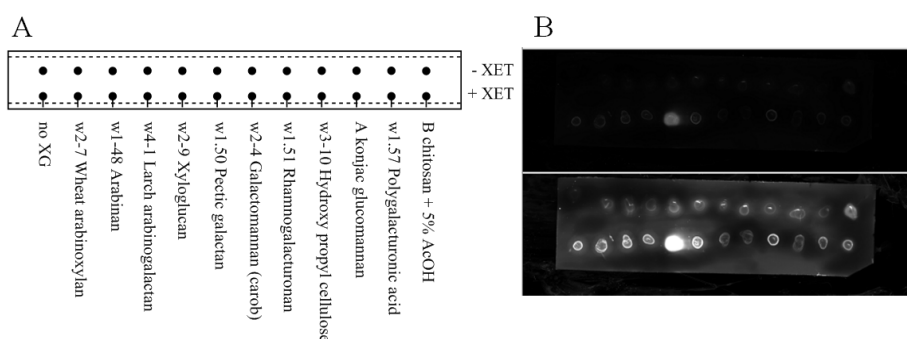
**Figure 26 The fluorescently labeled xyloglucan XGO.**

XGO\* was incubated with XG from tamarind kernel powder and a variety of other polysaccharides, listed in Figure 27, all in the presence of the XET enzyme. Control experiments without XET were also performed.

After incubation, the samples were manually spotted onto nitrocellulose sheets, in the pattern illustrated in Figure 27 A, and blocked with BSA in PBS buffer.

Finally the sheet was washed with PBS solution, dried on filter paper, and scanned.

As the image of the nitrocellulose slide (Figure 27 B) illustrates, XET only fused XGO\* with XG and no other polysaccharides showed fluorescence after the incubation. Weak spots from the remaining samples are also observed in Figure 27 B, but that is mainly due to the scanning settings. The power settings adjust how much power the laser will use to excite the sample. A photo multiplier tube (PMT) converts the amount of light striking the detector into a numeric value.

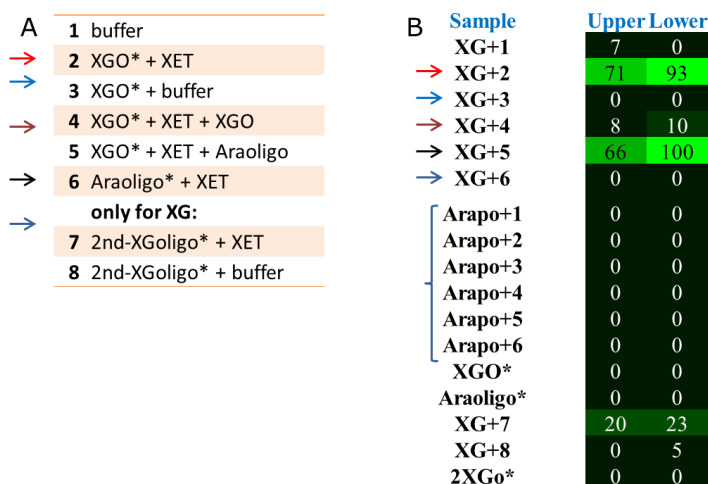


**Figure 27 Reaction between XGO and different polysaccharides with and without XET spotted on a nitrocellulose sheet.**

Reactions directly on the array were pursued, in which XG was spotted on nitrocellulose in different concentrations. The sheets were then incubated with a solution of XET and XGO in PBS buffer at 30 °C for 24 and 48 hours in darkness. A control experiment with XET was also performed. No difference was observed between the experiment and the controls, therefore this strategy was abandoned.

An Arrayjet printer was used to spot a different nitrocellulose sheet with different combinations of XG, XGO, XGO\*, XET, arabinopolysaccharide (Arapo), arabinopentaose (Araoligo) and a fluorescently labeled arabinohexasaccharide (Araoligo\*) synthesized by Beatrice Bonora. Figure 28 presents the results, in which A holds an overview of the incubation conditions for each polysaccharide (XG and Arapo) examined, and B presents the results as a heat map, in which the color intensities of each spot on the scanned array is related to a numeric value, where 100 represents the brightest spot. The scanned array and the location of each sample on the array is found in appendix B.





**Figure 28** Transglycosylase experiment printed on an Arrayjet printer. **A)** Overview of the incubation conditions used for each sample, with color codes referring to the heat map **B**. **B)** Heat map of the scanned array. Upper and lower refer to highest and lowest value in a triplicate set.

From Figure 28 we observe no signal, when only XG and buffer was printed (XG+1), but full signal was found for XG incubated with XGO\* and XET (XG+2). XET was essential in the reaction, as no signal was present when XG was incubated without XET (XG+3). In XG+4, the polysaccharide was incubated with XET, XGO and XGO\*, which clearly showed that XGO was a competitive inhibitor, whereas no inhibition was observed when Araoligo was added instead of XGO (XG-5). Incubating XG with XET and Araoligo\* also did not lead to any signal (XG+6), meaning that XET does not catalyze the transglycosylation between the two under the given conditions. Likewise, no transglycosylation activity was seen between Arapo and XGO\* in the presence of XET (Arapo+1-6). This suggests that the fluorescent XGO only identifies XG chains.

The XGO\* and 2XGO\* samples were two different batches of fluorescently labeled xyloglucan incubated in PBS buffer as control experiments. Araoligo\* incubated with PBS buffer were also spotted as a control experiment. All the non-linked sugars gave negative signals because of their inability to stick to the nitrocellulose surface. Sample XG+7 showed XG after incubation with 2XGO\* and XET, the results suggested that the fluorescent xyloglucan from the first batch for some reason was the most potent.

### 4.2.1 Perspective

Encouraged by the obtained results, it would be interesting to examine the possibility of identifying new transglycosylases with this assay. A fluorescently labeled oligosaccharide and a polysaccharide of the same sugar should be incubated with different plant tissue and the mixtures should be spotted onto a nitrocellulose sheet and examined under fluorescent light. In case of a signal, a transglycosylase, catalyzing the reaction between the two sugars, would be present in the plant tissue.

A carbohydrate microarray-based method to screen transglycosylase activity has been reported before by Kosík *et al.*<sup>200</sup> In their study they successfully printed the polysaccharide substrates (putative donors) onto nitrocellulose coated slides and the printed arrays were afterwards incubated with fluorescently labelled oligosaccharide (putative acceptor) and enzymatic samples. They used the MicroGrid printer. However the present study is novel in the sense of having the enzyme incubation with both substrates (acceptor and donor) in solution and printing the digestion mixtures afterwards. This method is believed to be superior, giving a lot fewer false negatives, as the performance of enzymes on immobilized substrate currently is known not to perform well.

## 4.3 Experimental

### 4.3.1 Linkers

Oligosaccharides and enzymes were purchased from Megazyme. Linker **160** was purchased from ABCR.

The procedures described by Pedersen *et al.*<sup>8</sup> were applied with some modifications:

#### *BSA Conjugation Reaction*

The conjugation of oligosaccharides to BSA was done by colleagues in the Willats' group by the procedure described by Roy *et al.*<sup>185</sup>

#### *Small Linker Conjugations Reactions*

10 mg/mL solutions of each oligosaccharide in aqueous acetate buffer (0.1 M, pH: 4.9) were prepared. Linker **160** and linker **161** solutions were prepared in the same buffer at 11.2 mg/mL and 100 mg/mL, respectively.

50 µL oligosaccharide solution was mixed with 20 equivalents of linker, and the reactions were incubated at 37 °C for 48 hours and followed on TLC using sulfuric acid and ninhydrin as stains. No work-up procedure was performed for the *unpurified* samples.

#### *Purification of Linker-Sugar Conjugates*<sup>201</sup>

An “Extract Clean™ Carbograph” column was activated with an activating solution of 80 % CH<sub>3</sub>CN, 20% H<sub>2</sub>O, and 0.1% TFA, followed by equilibration with milliQ water. The sample was loaded onto the column, followed by two wash cycles with water, which eluted the linker in the first wash. The sugar-linker compound eluted last with 50% CH<sub>3</sub>CN in water. The CH<sub>3</sub>CN was evaporated by a stream of air, and the samples were concentrated using a freeze dryer.

#### *Reduction*

18 µL of the 40 mM linker-sugar conjugate solutions (= 720 µmol) were moved to new vials with screw caps and freeze dried overnight. Next morning 50 equiv. NaBH<sub>3</sub>CN (~2 mg) in 50 µL solutions (70% dimethyl sulfoxide (DMSO) and 30% glacial AcOH) was added to sugar-conjugate at 65 °C with shaking. After 3 hours, the samples were concentrated and the DMSO solution was added to 200 µL water and freeze dried over 48 hours.

#### *Microarray Printing*

Carbohydrate microarrays were printed using two types of microarray robots, a pin-based MicroGrid II (Digilab/Genomic Solutions, Huntingdon, UK) and a piezoelectric Sprint (Arrayjet, Roslin, UK).

The printed slides were stored in the -18 °C until used.

#### *Incubation with antibodies*

The slide was thawed, blocked with blocking buffer (50 mM ethanolamine, 50 mM sodium phosphate, pH = 9.2) for 20 minutes, washed twice with PBS buffer, put in a dividing box so each array on the plate could be treated differently. Primary antibodies (1 mAbs, working solutions from the fridge) were diluted 1:10 in PBS buffer and 200 µL of the solution was added to each array.

After 1 hour at room temperature, covered with parafilm, the primary antibody was removed and the slide was washed three times with PBS buffer. Then the arrays were treated with secondary antibody (diluted 1:250, 200 µL added to each array). The slide was kept dark and incubated for one hour before the secondary antibody was removed and the slide washed with PBS buffer three times. The slide was centrifuged to remove excess water.

When the slide was incubated with enzyme (1 hour at 40 °C at 100 rpm), this was done before treating it with antibodies.

#### *Scanning and Analysis*

Slides were scanned using a slide scanner (GenePix 4100, Molecular Devices, Sunnyvale, CA). The output was analyzed using microarray analysis software (ImaGene 6.0, BioDiscovery, El Segundo, CA).

### 4.3.2 Transglycosylases

4.2 mg XG from tamarind kernel powder was dissolved in 1.05 mL buffer (4g/L). 2.3 mg XGO\* was dissolved in 1.15 mL buffer (2 g/L). A 0.78 mg/mL solution of XET was delivered from Novozymes.

50  $\mu$ L XG, 50  $\mu$ L XGO and 128  $\mu$ L XET were incubated at 1400 rpm at 30  $^{\circ}$ C covered by aluminum foil. After end reaction time the samples were heated to 75  $^{\circ}$ C for 10-15 min to denaturize the enzyme. The solution was centrifuged at 12000g for 20 min at 4  $^{\circ}$ C to remove denatured XET. Following 1  $\mu$ L of the heated solution was spotted on a nitrocellulose sheet.

*Arrayjet printed Array:*

Expanded version of the conditions in Figure 28A.

- 
- |          |  |
|----------|--|
| <b>1</b> | 15 $\mu$ L buffer  |
| <b>2</b> | 10 $\mu$ L 1mg/mL XGO* + 15 $\mu$ L XET                            |
| <b>3</b> | XGO* + 15 $\mu$ L buffer   |
| <b>4</b> | 5 $\mu$ L 2mg/mL XGO* + 15 $\mu$ L XET + 5 $\mu$ L 2mg/mL XGO      |
| <b>5</b> | 5 $\mu$ L 2mg/mL XGO* + 15 $\mu$ L XET + 5 $\mu$ L 2mg/mL Araoligo |
| <b>6</b> | 10 $\mu$ L 1mg/mL Araoligo* + 15 $\mu$ L XET                       |
|          | <b>only for XG:</b>  |
| <b>7</b> | 10 $\mu$ L 1mg/mL 2nd-XGoligo* + 15 $\mu$ L XET                    |
| <b>8</b> | 10 $\mu$ L 1mg/mL 2nd-XGoligo* + 15 $\mu$ L buffer                 |
-

## 5 Conclusion

The aim of the project was to develop an efficient and reliable strategy for synthesizing defined fragments of arabinofuranosides found in plant cell wall pectic polysaccharides. A general method for preparation of differentially protected hexasaccharide backbones with branching potentials is the major outcome of the project. The strategy utilizes a convergent block strategy where a disaccharide donor was used as building block to reduce the required number of glycosylation reactions. Furthermore, late stage selective deprotection made it possible to synthesize branched arabinans, which have not previously been described.

A broad range of synthetic routes towards a (1→5)-linked  $\alpha$ -diarabinoside donor was studied (chapter 3.3). Due to the high reactivity of arabinofuranosides, the most efficient route to a disaccharide donor employed a perbenzoylated monosaccharide serving as glycosyl donor. Three monosaccharide donors with a chloroacetyl group as alternative protecting group were also prepared. The following coupling of these donors in different combinations resulted in three different hexasaccharide backbone structures. The chloroacetyl group employed in different positions of the fourth monosaccharide of the hexasaccharides was removed selectively, converting the hexasaccharides into the corresponding glycosyl acceptors. Two of the hexasaccharides were further branched through reaction with a diarabinofuranoside donor to furnish two branched octasaccharides.

The major challenge faced throughout the syntheses was the selective removal of one protecting group from the different mono- and oligosaccharide intermediates. Final hydrogenolysis of the anomeric benzyl group also proved to be a challenge.

In conclusion, this constitutes the first reported synthesis of (1→5)-linked  $\alpha$ -arabinans branched at the 2- or the 3-position within the chain. In order to harness the full potential of the target molecules and apply them in the study of for example protein-carbohydrate interactions, deprotection remains, which we believe should be achievable using Zemplén conditions.

Furthermore, a linker system utilizing small molecules to link oligosaccharides to an array surface has been investigated. The small linkers were found to be superior to the use of neoglycoconjugates of bovine serum albumin, when studying enzyme activity. This was demonstrated by head-to-head comparison,

where five glycosyl hydrolases were able to react with the oligosaccharides immobilized on the array surface using the small linker, but not with the same polysaccharides conjugated to BSA.

Finally, an assay to screen for novel glycosyl transferase/hydrolase activities was developed. The studies showed that it was possible to detect transglycosylation activity on a microarray by spotting solutions of polyxyloglucan incubated with a fluorescently labeled heptaxyloglucan and glycosyltransferase, XET. These results suggest the potential of identifying new transglycosylases with this assay.

## 6 Experimental

### 6.1 General Comments

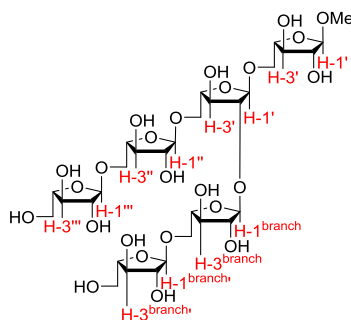
Starting materials, reagents, and solvents were purchased from commercial suppliers and were used without further purification. All solvents are HPLC grade. All reactions under argon or nitrogen atmosphere were performed in oven- or flame-dried glassware and with dry solvents. Anhydrous THF,  $\text{CH}_2\text{Cl}_2$ , and DMF were obtained from Innovative Technology PS-MD-7 Pure-solv solvent purification system. Anhydrous pyridine was obtained from refluxing pyridine with  $\text{CaH}_2$  approximate two hours before distilling.

Reactions were monitored by TLC, performed on Merck Aluminium Sheets pre-coated with silica, C-60 F<sub>254</sub> plates. Compounds were visualized with UV light (254 nm) and/or by heating after treatment with CAN stain ( $\text{Ce}(\text{SO}_4)_2$  (1.6 g) and  $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$  (4 g) in 10% sulphuric acid (200 mL)). Eluent systems are specified for each  $R_f$ -value, and ratios are given as volume ratios. Evaporation of solvents was performed with a VWR International Laborota 400 under reduced pressure (*in vacuo*) at temperatures ranging between 20-55 °C. Trace solvent was removed under reduced pressure by means of an oil pump. Flash chromatography was performed using Matrex 60 Å silica gel (35-70 µm) as the stationary phase. The eluent system is specified in the protocol for each synthesis. Eluent ratios are given as volume ratios. Automated flash chromatography was performed using a Teledyne Isco Combiflash Rf200 and Redisep Rf Gold Silica columns. All columns were treated with a gradient of heptane-EtOAc from 0% to 100% EtOAc. The intervals indicated for the eluent system under each entry implies where the compound eluted. Dry column vacuum chromatography was performed using Matrex 60 Å silica gel (15-40 µm) as the stationary phase.

All new compounds were characterized by  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR, infrared (IR) spectroscopy, high-resolution mass spectroscopy (HRMS) (electron spray ionization (ESI)), melting point, and optical rotation. NMR-spectra were recorded on a Bruker Ascend 400 MHz, Varian Mercury 300 MHz, or Bruker AC 500 MHz spectrometer at 298 K. Chemical shifts ( $\delta$ ) are reported in parts per million (ppm), coupling constants ( $J$ ) in Hz, and the field is indicated in each case. The solvent was  $\text{CDCl}_3$  and its resonance was used as internal standard ( $^1\text{H}$  7.26 ppm,  $^{13}\text{C}$  77.16 ppm).  $^{13}\text{C}$  NMR spectra were  $^1\text{H}$  decoupled and reported without discrimination of intensities in the aromatic region. All NMR spectra are assigned, however the protons on free hydroxyl groups are

generally not assigned. H-1 refers to the proton attached to C-1 of the monosaccharide in the reducing end of the glycoside. H-1' refers to the proton attached to the first carbon of the next monosaccharide, named C-1', and so on. In the case of branching, the proton attached to the C-1 closest to the core hexasaccharide is recorded as H-1<sup>branch</sup>, and H-1 in the next monosaccharide in the branching is noted H-1<sup>branch'</sup>, see Figure 29 for an example, in which the H-1 and H-3s are marked in red. For some of the larger oligosaccharide structures it was not possible to distinguish between the specific positions of certain protons in the chain. In these cases the protons in question are marked with a question mark instead of a number of primes, e.g. H-4<sup>?</sup>. Multiplicities of NMR signals are reported as: singlet (s), broad singlet (br), doublet (d), doublet of doublets (dd), doublet of doublet of doublets (ddd), doublet of triplets (dt), triplet (t), triplet of doublets (td), quartet (q), multiplet (m), and AB spin systems (AB spin). The AB spin systems in this thesis are reported with the two calculated chemical shifts of the two doublets separated by a comma followed by characterization of the peaks: 4.07, 4.03 (AB spin,  $J_{AB} = 15.2$  Hz, 2H, CH<sub>2</sub>Cl). The chemical peaks are calculated from the following formula

$$\delta_A = \frac{v_{center} + \frac{1}{2}\sqrt{(v_1 - v_4)(v_2 - v_3)}}{MHz} \qquad \delta_B = \frac{v_{center} - \frac{1}{2}\sqrt{(v_1 - v_4)(v_2 - v_3)}}{MHz}$$



**Figure 29** Illustration of how the protons are named in the NMR assignment of the synthesized compounds.

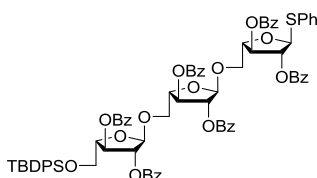
IR analysis was done on a Bruker Alpha-P FT-IR instrument where solid compound is applied directly to the instrument. Optical rotation was measured on a Perkin-Elmer 341 Polarimeter (cuvette 1.0 mL, 100 mm), using CHCl<sub>3</sub> as solvent using a sodium-source lamp (589 nm, 23 °C). Melting points (mp) were measured on a Stuart melting point SMP30 and reported in °C uncorrected. HRMS spectra were performed on an Agilent 1100 system equipped with a photodiode array detector and coupled to an LCT orthogonal time-of-flight



mass spectrometer (Waters-Micromass, Manchester, UK) with Z-spray electrospray ionization source and a LockSpray probe and controlled using MassLynx 4.0 software.

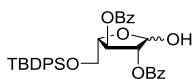
## 6.2 Procedures

**(85) Phenyl (2,3-di-*O*-benzoyl-5-*O*-(*tert*-butyldiphenylsilyl)- $\alpha$ -L-arabinofuranosyl)-(1 $\rightarrow$ 5)-(2,3-di-*O*-benzoyl- $\alpha$ -L-arabinofuranosyl)-(1 $\rightarrow$ 5)-2,3-di-*O*-benzoyl-1-thio- $\alpha$ -L-arabinofuranoside**



Compound **85** was obtained as a fluffy white solid as a byproduct in the synthesis of compound **99**.

**R<sub>f</sub>** 0.60 (10:1 toluene-EtOAc); [ $\alpha$ ]<sub>D</sub><sup>20</sup> - 35.7 (*c* 1.0 CHCl<sub>3</sub>); **mp.** 74.0 - 80.6 °C. **<sup>1</sup>H NMR** (500 MHz, CDCl<sub>3</sub>)  $\delta$  8.09 (dd, *J* = 8.3, 7.1 Hz, 2H), 8.04 - 7.96 (m, 5H), 7.95 - 7.90 (m, 4H), 7.74 - 7.68 (m, 4H), 7.63 - 7.20 (m, 30H), 5.82 (br, 1H, H-1), 5.77 (d, *J* = 4.3 Hz, 1H, H-3), 5.74 (t, *J* = 1.5 Hz, 1H, H-2), 5.68 - 5.63 (m, 3H, H-2', H-3', H-3''), 5.58 (d, *J* = 0.9 Hz, 1H, H-2''), 5.41 - 5.40 (m, 2H, H-1', H-1''), 4.72 (q, *J* = 4.3 Hz, 1H, H-4), 4.64 (q, *J* = 4.0 Hz, 1H, H-4'), 4.52 (q, *J* = 4.6 Hz, 1H, H-4''), 4.27 (dd, *J* = 11.3, 4.3 Hz, 1H, H-5a), 4.19 (dd, *J* = 11.3, 4.0 Hz, 1H, H-5'a), 4.02 - 3.91 (m, 4H, H-5b, H-5'b, H-5''a+b), 1.03 (s, 9H, C-(CH<sub>3</sub>)<sub>3</sub>) ppm. **<sup>13</sup>C NMR** (101 MHz, CDCl<sub>3</sub>)  $\delta$  165.8, 165.6 (2C), 165.4, 165.3, 165.3, 135.8, 135.8, 134.0, 133.6, 133.5, 133.4, 133.3, 133.2, 132.0, 130.1, 130.1, 130.0, 129.9, 129.9, 129.8, 129.4, 129.4, 129.3, 129.2, 129.0, 128.7, 128.6, 128.5, 128.4, 128.4, 127.8, 127.7, 106.1 (2C, C-1', C-1''), 91.4 (C-1), 83.3 (C-4''), 82.3 (4C, C-2, C-2'', C-4, C-4'), 81.7 (C-2'), 77.4 (3C, C-3, C-3', C-3''), 65.9 (C-5'), 65.8 (C-5), 63.5 (C-5''), 26.9 (C-(CH<sub>3</sub>)<sub>3</sub>), 19.4 (C-(CH<sub>3</sub>)<sub>3</sub>) ppm. **IR** (neat, cm<sup>-1</sup>): 3069, 2930, 2857, 1719, 1451, 1248, 1094, 704. **ESIMS**: *m/z* calcd for [C<sub>79</sub>H<sub>72</sub>NaO<sub>18</sub>SSi]<sup>+</sup>: 1391.4101 [M+Na]<sup>+</sup>, found: 1391.4099.

**(86) 2,3-Di-*O*-benzoyl-5-*O*-*tert*-butyldiphenylsilyl-L-arabinofuranose**

A solution of compound **103** (5 g, 7.26 mmol) in acetone (45 mL) was cooled to 0 °C in an ice bath. Water (5 mL) and NBS (5.5 g, 30.9 mmol) were added and the reaction was stirred at 21 °C. The reaction came to completion after 1 hour. Afterwards solid NaHCO<sub>3</sub> (2 g) was added and the mixture was stirred for 20 minutes, before it was concentrated to remove most of the acetone. The resulting mixture was diluted with EtOAc (10 mL). The two phases were separated and the aqueous phase was washed with EtOAc (10 mL). The combined organic phases were washed with water (2 x 10 mL) and brine (10 mL), dried (MgSO<sub>4</sub>) and concentrated to give 6 g of a clear oil that was purified by column chromatography (1:4 heptane-EtOAc) to afford compound **86** (in a 1:0.8 mixture of the β:α mixture) as a colorless oil (3.6 g, 81%). The analytical data is in accordance with reported data for compound D-**86** *R<sub>f</sub>* 0.26 (4:1 hexane-EtOAc).<sup>138</sup>

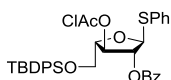
*R<sub>f</sub>* 0.47 (2:1 heptane-EtOAc).

α: <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 8.12 - 8.02 (m, 2H), 8.01 - 7.94 (m, 1H), 7.85 - 7.78 (m, 1H), 7.75 - 7.68 (m, 2H), 7.64 - 7.53 (m, 2H), 7.51 - 7.29 (m, 8H), 5.64 (dd, *J* = 4.7, 1.7 Hz, 0.8H, H-3), 5.61 (s, 0.8H, H-1), 5.48 (d, *J* = 1.7 Hz, 0.8H, H-2), 4.57 (q, *J* = 4.7 Hz, 0.8H, H-4), 3.99 (d, *J* = 4.7 Hz, 1.6H, H-5a+b), 1.04 (s, 7H, C-(CH<sub>3</sub>)<sub>3</sub>) ppm. <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) δ 166.3, 165.8, 136.2, 135.8, 133.7, 133.6, 133.4, 132.2, 131.9, 130.5, 130.2, 130.1, 129.9, 129.5, 129.4, 129.3, 128.7, 128.6, 128.2, 101.3 (C-1), 83.6 (C-4), 83.0 (C-2), 77.8 (C-3), 63.8 (C-5), 27.0 (C-(CH<sub>3</sub>)<sub>3</sub>), 19.5 (C-(CH<sub>3</sub>)<sub>3</sub>) ppm.

β: <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 8.12 - 8.02 (m, 4H), 8.01 - 7.94 (m, 1H), 7.75 - 7.68 (m, 3H), 7.64 - 7.53 (m, 2H), 7.51 - 7.29 (m, 10H), 6.03 (dd, *J* = 5.7, 4.0 Hz, 1H, H-3), 5.71 (br, 1H, H-1), 5.56 (dd, *J* = 5.7, 4.8 Hz, 1H, H-2), 4.28 - 4.24 (m, 1H, H-4), 4.08 (dd, *J* = 11.2, 2.9 Hz, 1H, H-5a), 3.91 (dd, *J* = 11.2, 2.4 Hz, 1H, H-5b), 1.12 (s, 9H, C-(CH<sub>3</sub>)<sub>3</sub>) ppm. <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) δ 166.0, 165.9, 135.9, 133.7, 133.6, 133.4, 132.2, 130.5, 130.3, 130.2, 130.1, 129.9, 129.5, 129.4, 129.3, 128.7, 128.6, 127.9, 95.6 (C-1), 83.1 (C-4), 79.6 (C-2), 76.6 (C-3), 65.2 (C-5), 27.1 (C-(CH<sub>3</sub>)<sub>3</sub>), 19.5 (C-(CH<sub>3</sub>)<sub>3</sub>) ppm.

**IR** (neat,  $\text{cm}^{-1}$ ): 3459, 3071, 2957, 2930, 2857, 1722, 1451, 1262, 1106, 1067, 1026, 996, 700, 503. **ESIMS**:  $m/z$  calcd for  $[\text{C}_{35}\text{H}_{36}\text{NaO}_7\text{Si}]^+$ : 619.2122  $[\text{M}+\text{Na}]^+$ , found: 619.2128.

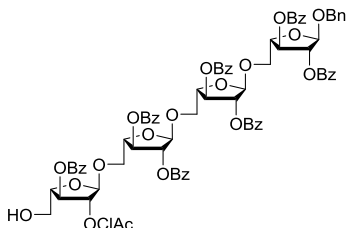
**(87) Phenyl 2-*O*-benzoyl-5-*O*-*tert*-butyldiphenylsilyl-3-*O*-chloroacetyl-1-thio- $\alpha$ -L-arabinofuranoside**



Thioglycoside **153** (2.90 g, 4.95 mmol) was dissolved in anhydrous DMF (60 mL).  $\text{NaHCO}_3$  (0.62 g, 7.43 mmol) and chloroacetic anhydride (1.32 g, 7.92 mmol) were added. TLC indicated that the reaction was complete after 4.5 hours. The reaction mixture was washed with a solution of sat. aq.  $\text{NaHCO}_3$  (100 mL) and water (3 x 100 mL), then it was dried ( $\text{MgSO}_4$ ) and concentrated before column chromatographed in 1:0  $\rightarrow$  2:1 (heptane-EtOAc) to get 3.04 g colorless oil. The title product was obtained in 92% yield.

$R_f$  0.19 (10:1 heptane-EtOAc),  $[\alpha]_D^{20}$  -68.8 ( $c$  0.90  $\text{CHCl}_3$ ).  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  7.95 - 7.86 (m, 2H), 7.65 - 7.57 (m, 4H), 7.54 - 7.43 (m, 3H), 7.39 - 7.20 (m, 11H), 5.64 (d,  $J = 2.5$  Hz, 1H, H-1), 5.51 (dd,  $J = 5.4$ , 2.5 Hz, 1H, H-3), 5.44 (t,  $J = 2.5$  Hz, 1H, H-2), 4.41 - 4.36 (m, 1H, H-4), 4.07, 4.03 (AB spin,  $J_{AB} = 15.2$  Hz, 2H,  $\text{CH}_2\text{Cl}$ ), 3.89 (d,  $J = 4.2$  Hz, 2H, H-5a+b), 0.98 (s, 9H, C-( $\text{CH}_3$ )<sub>3</sub>) ppm.  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  166.6, 165.5, 135.8, 134.9, 133.7, 133.6, 133.2, 133.1, 133.0, 132.3, 130.1, 130.0, 129.9, 129.8, 129.2, 129.0, 128.6, 127.9, 90.6 (C-1), 82.4 (C-4), 82.3 (C-2), 78.7 (C-3), 63.2 (C-5), 40.8 ( $\text{CH}_2\text{Cl}$ ), 26.9 (C-( $\text{CH}_3$ )<sub>3</sub>), 19.4 (C-( $\text{CH}_3$ )<sub>3</sub>) ppm. **IR** (neat,  $\text{cm}^{-1}$ ): 3071, 2956, 2930, 2857, 1726, 1427, 1264, 1106, 1026, 822, 740, 700, 606, 503. **ESIMS**:  $m/z$  calcd for  $[\text{C}_{36}\text{H}_{37}\text{ClNaO}_6\text{SSi}]^+$ : 683.1661  $[\text{M}+\text{Na}]^+$ , found: 683.1661.

**(88) Benzyl (3-*O*-benzoyl-2-*O*-chloroacetyl- $\alpha$ -L-arabinofuranosyl)-(1 $\rightarrow$ 5)-(2,3-di-*O*-benzoyl- $\alpha$ -L-arabinofuranosyl)-(1 $\rightarrow$ 5)-(2,3-di-*O*-benzoyl- $\alpha$ -L-arabinofuranosyl)-(1 $\rightarrow$ 5)-2,3-di-*O*-benzoyl- $\alpha$ -L-arabinofuranoside**

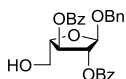


Compound **143** (200 mg, 0.119 mmol) was dissolved in  $\text{CH}_2\text{Cl}_2$  (2 mL) and MeCN (6 mL) in a plastic flask. HF (0.155 mL, 70% in pyridine) and one drop of water were added and the reaction was stirred at 22 °C for 24 hours.  $\text{Me}_3\text{SiOMe}$  (0.900 mL, 6.3 mmol) was added to quench the reaction and the mixture was stirred for 20 minutes before it was diluted with EtOAc (3 mL), washed with a solution of sat. aq.  $\text{NaHCO}_3$  (2 x 25 mL) and water (25 mL), and dried ( $\text{Na}_2\text{SO}_4$ ). The organic solution was concentrated to afford 600 mg of a fluffy white solid that was dispersed in 2.5 g silica and purified on a 12 g column by automated flash chromatography (heptane-EtOAc, 40%  $\rightarrow$  45% EtOAc). The title compound was obtained as a white solid in 70% yield (120 mg).

$R_f$  0.21 (1:1 heptane-EtOAc);  $[\alpha]_D^{20}$  -28.4 ( $c$  0.99  $\text{CHCl}_3$ ); **mp.** 83.8 - 85.4 °C.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  8.04 - 7.97 (m, 8H), 7.93 - 7.83 (m, 6H), 7.59 - 7.47 (m, 4H), 7.46 - 7.35 (m, 12H), 7.35 - 7.21 (m, 11H), 5.64 (s, 2H, H-3, H-3'), 5.63 (s, 1H, H-2<sup>3</sup>), 5.61 (d,  $J$  = 1.0 Hz, 1H, H-2<sup>3</sup>), 5.59 (d,  $J$  = 1.1 Hz, 1H, H-2<sup>3</sup>), 5.57 (d,  $J$  = 4.9 Hz, 1H, H-3''), 5.44 (d,  $J$  = 1.6 Hz, 1H, H-2'''), 5.39 (s, 2H, H-1', H-1''), 5.32 (s, 1H, H-1), 5.30 - 5.26 (m, 2H, H-1''', H-3'''), 4.83 (d,  $J$  = 12.0 Hz, 1H,  $\text{CH}_2\text{Ph}$ ), 4.62 - 4.54 (m, 3H,  $\text{CH}_2\text{Ph}$ , H-4, H-4'), 4.47 (td,  $J$  = 4.6, 1.9 Hz, 1H, H-4'), 4.40 (dt,  $J$  = 5.0, 3.6 Hz, 1H, H-4'''), 4.22 - 4.04 (m, 5H, 3 x H-5a,  $\text{CH}_2\text{Cl}$ ), 3.96 - 3.89 (m, 4H, 3 x H-5b, H-5'''a), 3.87 - 3.81 (m, 1H, H-5'''b) ppm.  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$  166.2, 166.1, 165.8 (3C), 165.5, 165.3, 165.2, 137.5, 133.5, 133.4, 130.1, 130.0, 129.9, 129.9, 129.2, 128.6, 128.6, 128.5, 128.5, 128.4, 128.4, 127.9, 105.9, 105.9 (C-1', C-1''), 105.5 (C-1'''), 105.0 (C-1), 83.2 (C-4'''), 82.9 (C-2'''), 82.3, 82.2 (C-4, C-4'), 82.0 (C-4''), 81.9 (C-2<sup>3</sup>), 81.8 (C-2<sup>3</sup>), 81.7 (C-2<sup>3</sup>), 77.4 (3C, C-3, C-3', C-3''), 77.2 (C-3'''), 68.7 ( $\text{CH}_2\text{Ph}$ ), 66.2 (C-5<sup>3</sup>), 66.1 (C-5<sup>3</sup>), 66.0 (C-5<sup>3</sup>), 62.0 (C-5'''), 40.7 ( $\text{CH}_2\text{Cl}$ ) ppm. **IR** (neat,  $\text{cm}^{-1}$ ): 2929,

1716, 1601, 1451, 1248, 1106, 1024, 706. **ESIMS:**  $m/z$  calcd for  $[C_{78}H_{69}ClNaO_{25}]^+$ : 1463.3709  $[M+Na]^+$ , found: 1463.3706.

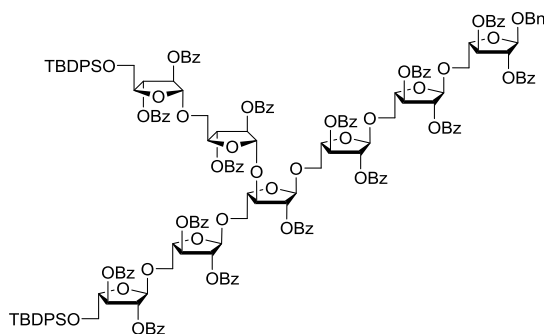
**(89) Benzyl 2,3-di-*O*-benzoyl- $\alpha$ -L-arabinofuranoside**



Compound **107** (9.96 g, 14.5 mmol) was dissolved in anhydrous THF (100 mL) and cooled to 0 °C in an ice bath. Acetic acid (1.62 mL, 28.4 mmol) and TBAF (1 M in THF) (19.2 mL, 19.2 mmol) were added to the reaction mixture. The reaction was stirred for 24 h at 4 °C. After completion of the reaction the mixture was diluted with  $CH_2Cl_2$  (5 mL) and washed with a solution of sat. aq.  $NH_4Cl$  (2 x 200 mL) and water (200 mL). The organic phase was dried ( $MgSO_4$ ) and concentrated to dryness. The resulting colorless oil was purified on silica gel (4:1 heptane-EtOAc) to afford compound **89** as a colorless oil (5.54 g, 84%).

$R_f$  0.19 (2:1 heptane-EtOAc),  $[\alpha]_D^{20}$  - 2.9 ( $c$  1.0,  $CHCl_3$ ).  **$^1H$  NMR** (300 MHz,  $CDCl_3$ )  $\delta$  8.10 - 8.01 (m, 4H), 7.59 (t,  $J$  = 7.4 Hz, 2H), 7.50 - 7.38 (m, 6H), 7.38 - 7.27 (m, 3H), 5.62 (s, 1H, H-2), 5.64 (d,  $J$  = 4.2 Hz, 1H, H-3), 5.35 (s, 1H, H-1), 4.86 (d,  $J$  = 11.9 Hz, 1H,  $CH_2Ph$ ), 4.64 (d,  $J$  = 11.9 Hz, 1H,  $CH_2Ph$ ), 4.36 (q,  $J$  = 4.2 Hz, 1H, H-4), 4.03 (dd,  $J$  = 12.0, 4.2 Hz, 1H, H-5a), 3.99 (dd,  $J$  = 12.0, 4.2 Hz, 1H, H-5b) ppm.  **$^{13}C$  NMR** (75 MHz,  $CDCl_3$ )  $\delta$  166.3, 165.4, 137.4, 133.7, 130.1, 130.0, 129.3, 129.2, 128.7, 128.6, 128.6, 127.9, 127.9, 104.9 (C-1), 84.1 (C-4), 81.9 (C-2), 78.0 (C-3), 68.9 ( $CH_2Ph$ ), 62.6 (C-5) ppm. **ESIMS:**  $m/z$  calcd for  $[C_{26}H_{24}NaO_7]^+$ : 471.1414  $[M+Na]^+$ , found: 471.1416.

**(94) Benzyl (2,3-di-*O*-benzoyl-5-*O*-(*tert*-butyldiphenylsilyl)- $\alpha$ -L-arabinofuranosyl)-(1 $\rightarrow$ 5)-(2,3-di-*O*-benzoyl- $\alpha$ -L-arabinofuranosyl)-(1 $\rightarrow$ 5)-[2,3-di-*O*-benzoyl-5-*O*-(*tert*-butyldiphenylsilyl)- $\alpha$ -L-arabinofuranosyl-(1 $\rightarrow$ 5)-2,3-di-*O*-benzoyl- $\alpha$ -L-arabinofuranosyl-(1 $\rightarrow$ 3)]-(2-*O*-benzoyl- $\alpha$ -L-arabinofuranosyl)-(1 $\rightarrow$ 5)-(2,3-di-*O*-benzoyl- $\alpha$ -L-arabinofuranosyl)-(1 $\rightarrow$ 5)-(2,3-di-*O*-benzoyl- $\alpha$ -L-arabinofuranosyl)-(1 $\rightarrow$ 5)-2,3-di-*O*-benzoyl- $\alpha$ -L-arabinofuranoside**

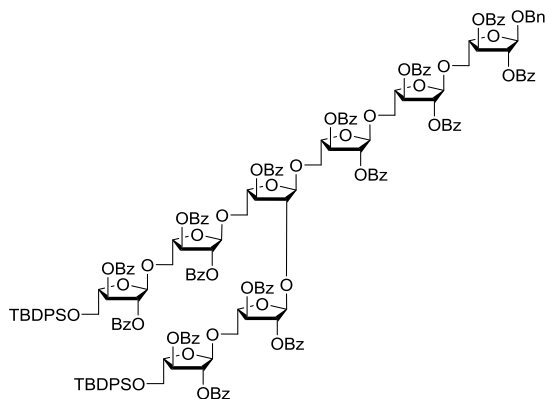


A mixture of hexasaccharide **148** (84 mg, 37  $\mu$ mol) and disaccharide donor **99** (51 mg, 49  $\mu$ mol) were coevaporated with toluene and dried under vacuum for 16 hours. The starting materials were dissolved in anhydrous  $\text{CH}_2\text{Cl}_2$  (2 mL) under  $\text{N}_2$  and stirred with 200 mg molecular sieves (4 Å) and NIS (11 mg, 49  $\mu$ mol) at 22 °C for 30 minutes. The reaction mixture was cooled in a – 45 °C bath and TfOH (0.8  $\mu$ L, 10  $\mu$ mol) was added. The reaction came to completion within 2.5 hours, under which the temperature was raised to – 35 °C. The molecular sieves were filtered off and the reaction mixture was poured into a mixture of 10% aq.  $\text{Na}_2\text{S}_2\text{O}_3$  (1 mL) and a solution of sat. aq.  $\text{NaHCO}_3$  (1 mL). The organic layer was isolated and washed with water (2 x 2 mL), dried ( $\text{Na}_2\text{SO}_4$ ), filtered and concentrated to give 374 mg of a yellow oil that was dispersed on 2.5 g silica and subjected to automated flash chromatography (heptane-EtOAc, 30%  $\rightarrow$  35% EtOAc) on a 4 g column. The title product was obtained as 71 mg fluffy white solid (60% yield).

$R_f$  0.42 (1:1 heptane-EtOAc);  $[\alpha]_D^{20}$  – 19.7 (*c* 0.96  $\text{CHCl}_3$ ); **mp.** 97.8 – 99.1 °C.  **$^1\text{H}$  NMR** (400 MHz,  $\text{CDCl}_3$ )  $\delta$  8.06 – 7.97 (m, 8H), 7.97 – 7.80 (m, 22H), 7.70 – 7.62 (m, 8H), 7.56 – 7.44 (m, 6H), 7.44 – 7.14 (m, 55H), 5.64 – 5.57 (m, 10H, 7  $\times$  H-3, 3  $\times$  H-2), 5.53 (d,  $J$  = 0.8 Hz, 1H, H-2 $^3$ ), 5.52 (d,  $J$  = 1.0 Hz, 1H, H-2 $^{3''}$ ), 5.51 – 5.49 (m, 2H, H-2 $^{3''}$ , H-1 $^3$ ), 5.48 (d,  $J$  = 1.2 Hz, 1H, H-2 $^3$ ), 5.44 (d,  $J$  = 1.2 Hz, 1H, H-2 $^3$ ), 5.40 (s, 1H, H-1 $^3$ ), 5.39 (s, 1H, H-1 $^3$ ), 5.37 (s, 1H, H-1 $^{3''}$ ), 5.32 (s, 1H, H-1), 5.30 (s, 1H, H-1 $^3$ ), 5.29 (s, 1H, H-1 $^3$ ), 5.21 (s,

$^1\text{H}$ ,  $\text{H-1}^?$ , 4.83 (d,  $J = 11.9$  Hz, 1H,  $\text{CH}_2\text{Ph}$ ), 4.63 - 4.57 (m, 2H,  $\text{CH}_2\text{Ph}$ ,  $\text{H-4}^?$ ), 4.56 - 4.52 (m, 1H,  $\text{H-4}^?$ ), 4.51 (d,  $J = 1.0$  Hz, 1H,  $\text{H-3}''''$ ), 4.50 - 4.40 (m, 5H,  $\text{H-4}^?$ ), 4.40 - 4.36 (m, 1H,  $\text{H-4}^?$ ), 4.19 (dd,  $J = 11.2, 4.6$  Hz, 2H,  $2 \times \text{H-5a}^?$ ), 4.16 - 4.08 (m, 3H,  $3 \times \text{H-5a}$ ), 4.01 (dd,  $J = 11.5, 4.0$  Hz, 1H,  $\text{H-5a}^?$ ), 3.98 - 3.85 (m, 8H,  $\text{H-5}''''''_{\text{a+b}}$ ,  $\text{H-5}^{\text{branch}}_{\text{a+b}}$ ,  $4 \times \text{H-5b}$ ), 3.82 (dd,  $J = 11.4, 2.3$  Hz, 2H,  $2 \times \text{H-5b}^?$ ), 3.74 (dd,  $J = 11.2, 2.3$  Hz, 1H,  $\text{H-5b}^?$ ), 0.99 (s, 9H,  $\text{C-(CH}_3)_3$ ), 0.97 (s, 9H,  $\text{C-(CH}_3)_3$ ) ppm.  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$  165.9, 165.7 (2C), 165.7, 165.6, 165.6, 165.5, 165.5, 165.4, 165.4, 165.3, 165.3, 165.2, 165.1, 164.9, 137.6, 135.8, 133.6, 133.5, 133.4, 133.3, 133.3, 133.1, 130.1, 129.9, 129.9, 129.8, 129.4, 129.2, 128.6, 128.5, 128.5, 128.4, 128.3, 127.9, 127.8, 106.1 (3C,  $3 \times \text{C-1}$ ), 105.9 (3C,  $3 \times \text{C-1}$ ), 105.4 ( $\text{C-1}^?$ ), 105.0 ( $\text{C-1}$ ), 83.3 ( $\text{C-4}''''$ ), 83.1 (3C,  $3 \times \text{C-4}$ ), 82.8 ( $\text{C-2}''''$ ), 82.3 (2C,  $2 \times \text{C-4}$ ,  $2 \times \text{C-2}$ ), 82.2 (2C,  $2 \times \text{C-4}$ ), 81.9 (2C,  $2 \times \text{C-2}$ ), 81.8 ( $\text{C-2}^?$ ), 81.7 ( $\text{C-2}^?$ ), 81.6 ( $\text{C-2}^?$ ), 81.4 ( $\text{C-3}''''$ ), 77.4 (3C,  $3 \times \text{C-3}$ ), 77.3 (4C,  $3 \times \text{C-3}$ ), 68.7 ( $\underline{\text{CH}_2}\text{Ph}$ ), 66.1 (3C,  $3 \times \text{C-5}$ ), 65.8 (3C,  $3 \times \text{C-5}$ ), 63.4 (2C,  $\text{C-5}''''''$ ,  $\text{C-5}^{\text{branch}}$ ), 26.9 ( $\text{C-(CH}_3)_3$ ), 19.4 ( $\underline{\text{C}}\text{-(CH}_3)_3$ ) ppm. IR (neat,  $\text{cm}^{-1}$ ): 2929.78, 1718.75, 1451.51, 1247.73, 1095.31, 1024.72, 962.06, 704.49. ESIMS:  $m/z$  calcd for  $[\text{C}_{184}\text{H}_{168}\text{NaO}_{48}\text{Si}_2]^+$ : 3224.0136  $[\text{M}+\text{Na}]^+$ , found: 3224.0041.

**(95) Benzyl (2,3-di-*O*-benzoyl-5-*O*-(*tert*-butyldiphenylsilyl)- $\alpha$ -L-arabinofuranosyl)-(1 $\rightarrow$ 5)-(2,3-di-*O*-benzoyl- $\alpha$ -L-arabinofuranosyl)-(1 $\rightarrow$ 5)-[2,3-di-*O*-benzoyl-5-*O*-(*tert*-butyldiphenylsilyl)- $\alpha$ -L-arabinofuranosyl-(1 $\rightarrow$ 5)-2,3-di-*O*-benzoyl- $\alpha$ -L-arabinofuranosyl-(1 $\rightarrow$ 2)]-(3-*O*-benzoyl- $\alpha$ -L-arabinofuranosyl)-(1 $\rightarrow$ 5)-(2,3-di-*O*-benzoyl- $\alpha$ -L-arabinofuranosyl)-(1 $\rightarrow$ 5)-(2,3-di-*O*-benzoyl- $\alpha$ -L-arabinofuranosyl)-(1 $\rightarrow$ 5)-2,3-di-*O*-benzoyl- $\alpha$ -L-arabinofuranoside**

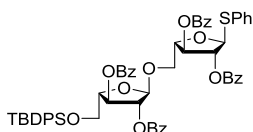


A mixture of hexasaccharide **150** (100 mg, 44  $\mu\text{mol}$ ) and disaccharide donor **99** (59 mg, 57  $\mu\text{mol}$ ) were coevaporated with toluene and dried under vacuum for 16 hours. The starting materials were dissolved in  $\text{CH}_2\text{Cl}_2$  (2 mL) under  $\text{N}_2$  and stirred with 200 mg molecular sieves (4 Å) and NIS (13 mg, 57  $\mu\text{mol}$ ) at 22 °C for 20 minutes. The reaction mixture was cooled in a – 55 °C bath and TfOH (1  $\mu\text{L}$ , 11  $\mu\text{mol}$ ) was added. The reaction seemed to come to completion within 4.5 hours, under which the temperature was raised to – 35 °C. The molecular sieves were filtered off and the reaction mixture was poured into a mixture of 10% aq.  $\text{Na}_2\text{S}_2\text{O}_3$  (1 mL) and a solution of sat. aq.  $\text{NaHCO}_3$  (1 mL). The organic layer was isolated and washed with water (2 x 2 mL), dried ( $\text{Na}_2\text{SO}_4$ ), filtered and concentrated to an yellow oil that was dispersed on 2.5 g silica and subjected to automated flash chromatography (heptane-EtOAc, 30%  $\rightarrow$  35% EtOAc) on a 4 g column. The title product was obtained as 17 mg fluffy white solid (12% yield).

$R_f$  0.42 (1:1 toluene-EtOAc);  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.93 - 7.84 (m, 12H), 7.79 - 7.65 (m, 11H), 7.57 - 7.07 (m, 63H), 5.80 - 5.75 (m, 2H, H-1<sup>branch</sup>, H-3<sup>branch</sup>), 5.68 - 5.58 (m, 9H, 6  $\times$  H-3, 3  $\times$  H-2), 5.58 - 5.52 (m, 4H, 4  $\times$  H-2), 5.43 (s, 1H, H-1'''), 5.39 (d,  $J$  = 3.8 Hz, 1H, H-3'''), 5.37 (s, 2H, H-1'', H-1'), 5.34 (s, 1H, H-1'), 5.32 (s, 2H, H-1, H-1'), 5.27 (s, 1H, H-1'), 4.83 (d,  $J$  = 11.9 Hz, 1H,  $\text{CH}_2\text{Ph}$ ), 4.72 - 4.67 (m, 1H, H-4'''), 4.63 - 4.53 (m, 4H,  $\text{CH}_2\text{Ph}$ , H-2''', 2  $\times$  H-4), 4.53 - 4.44 (m, 5H, 5  $\times$  H-4), 4.26 - 4.10 (m, 5H, H-5<sup>branch</sup><sub>a</sub>, 4  $\times$  H-5a), 4.06 (dd,  $J$  = 10.9, 5.6 Hz, 1H, H-5a''), 4.01 - 3.81 (m, 9H, H-5''', a+b, H-5<sup>branch</sup><sub>a</sub>+b, 5  $\times$  H-5b), 3.78 (dd,  $J$  = 10.9, 2.2 Hz, 1H, H-5<sup>branch</sup><sub>b</sub>), 1.00 (s, 18H, 2  $\times$  TBDPS) ppm.  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$  165.8 (2C), 165.7 (2C), 165.7 (2C), 165.6, 165.6, 165.5, 165.4, 165.3, 165.3, 165.2, 165.1, 164.9, 137.5, 135.8, 135.8, 133.4, 133.3, 133.2, 130.1, 130.1, 130.1, 130.0, 129.9, 129.8, 129.5, 129.4, 129.2, 128.6, 128.6, 128.5, 128.5, 128.4, 128.2, 127.9, 127.8, 106.3, 106.1, 106.1, 105.9, 105.9 (6  $\times$  C-1'), 105.0 (C-1), 104.5 (C-1<sup>branch</sup>), 83.3, 83.3 (C-2''', C-4<sup>branch</sup>), 82.4, 82.3 (2C), 82.3 (2C), 82.2 (3C), 81.9 (2C), 81.7, 81.6 (3C), (7  $\times$  C-2, 7  $\times$  C-4), 77.9 (C-3'''), 77.4 (5C), 77.3 (6  $\times$  C-4), 77.0 (C-3<sup>branch</sup>), 68.7 ( $\text{CH}_2\text{Ph}$ ), 66.1 (2C), 65.9 (3C), 65.8 (2C) (6  $\times$  C-5), 63.5, 63.4 (C-5''', C-5<sup>branch</sup>'), 26.9 (2  $\times$  C-( $\text{CH}_3$ )<sub>3</sub>), 19.4 (2  $\times$  C-( $\text{CH}_3$ )<sub>3</sub>) ppm.



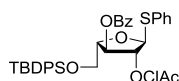
**(99) Phenyl (2,3-di-*O*-benzoyl-5-*O*-(*tert*-butyldiphenylsilyl)- $\alpha$ -L-arabinofuranosyl)-(1 $\rightarrow$ 5)-2,3-di-*O*-benzoyl-1-thio- $\alpha$ -L-arabinofuranoside**



A mixture of compound **104** (10 g, 22.2 mmol) and compound **102** (18.7 g, 26.6 mmol) were dried azeotropically with toluene and subjected to high vacuum for 16 hours. The flask was filled with Ar and the monosaccharides were dissolved in anhydrous  $\text{CH}_2\text{Cl}_2$  (300 mL). 22 g activated molecular sieves (4 Å) were added and the mixture was cooled in a  $-60\text{ }^\circ\text{C}$  bath. TMSOTf (5.02 mL, 30.7 mmol) was added dropwise over 30 minutes and the reaction was slowly heated to  $-50\text{ }^\circ\text{C}$ . The reaction came to completion after 6 hours according to TLC (2:1 heptane-EtOAc), then it was quenched with  $\text{Et}_3\text{N}$  (33 mL). The molecular sieves were filtered off and washed with  $\text{CH}_2\text{Cl}_2$  (5 mL). The combined organic phases were washed with a solution of sat. aq.  $\text{NaHCO}_3$  (200 mL) and water (200 mL), dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and concentrated. After subjecting the mixture to automated flash chromatography (heptane-EtOAc, 10%  $\rightarrow$  25% EtOAc) 19.4 g fluffy white solid was obtained as compound **99** (85%).

$R_f$  0.25 (6:1 heptane-EtOAc);  $[\alpha]_D^{20}$  -34.4 ( $c$  2.0  $\text{CHCl}_3$ ); **mp.** 59.6 - 60.3  $^\circ\text{C}$ .  $^1\text{H NMR}$  (500 MHz,  $\text{CDCl}_3$ )  $\delta$  8.13 - 8.07 (m, 2H), 7.96 (d,  $J$  = 8.1 Hz, 4H), 7.93 - 7.89 (m, 2H), 7.73 - 7.66 (m, 4H), 7.61 - 7.52 (m, 4H), 7.49 - 7.42 (m, 4H), 7.40 - 7.20 (m, 15H), 5.79 (s, 1H, H-1), 5.74 (d,  $J$  = 4.7 Hz, 1H, H-3), 5.71 (d,  $J$  = 1.4 Hz, 1H, H-2), 5.62 (d,  $J$  = 4.2 Hz, 1H, H-3'), 5.55 (d,  $J$  = 1.0 Hz, 1H, H-2'), 5.36 (s, 1H, H-1'), 4.73 (q,  $J$  = 4.2 Hz, 1H, H-4), 4.50 (q,  $J$  = 4.2 Hz, 1H, H-4'), 4.23 (dd,  $J$  = 11.1, 4.2 Hz, 1H, H-5a), 3.98 (d,  $J$  = 4.2 Hz, 2H, H-5'a+b), 3.96 (dd,  $J$  = 11.1, 4.2 Hz, 1H, H-5b), 1.01 (s, 9H, C-( $\text{CH}_3$ )<sub>3</sub>) ppm.  $^{13}\text{C NMR}$  (75 MHz,  $\text{CDCl}_3$ )  $\delta$  165.7 (2C), 165.5, 165.5, 135.9, 135.9, 134.1, 133.8, 133.7, 133.5, 133.5, 133.4, 133.3, 132.1, 130.2, 130.2, 130.0, 129.9, 129.4, 129.4, 129.3, 129.0, 128.8, 128.7, 128.6, 128.5, 127.9, 127.8, 106.2 (C-1'), 91.5 (C-1), 83.5 (C-4'), 82.4 (C-4), 82.4 (C-2'), 82.3 (C-2), 77.5 (C-3), 77.4 (C-3'), 65.9 (C-5), 63.5 (C-5'), 26.9 (C-( $\text{CH}_3$ )<sub>3</sub>), 19.4 (C-( $\text{CH}_3$ )<sub>3</sub>) ppm. **IR** (neat,  $\text{cm}^{-1}$ ): 3070, 2931, 2858, 1721, 1452, 1261, 1105, 1026, 755, 706. **ESIMS**:  $m/z$  calcd for  $[\text{C}_{60}\text{H}_{56}\text{NaO}_{12}\text{SSi}]^+$ : 1051.3154  $[\text{M}+\text{Na}]^+$ , found: 1051.3136.

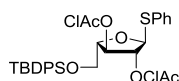
**(100) Phenyl 3-*O*-benzoyl-5-*O*-*tert*-butyldiphenylsilyl-2-*O*-chloroacetyl-1-thio- $\alpha$ -L-arabinofuranoside**



Compound **126** (630 mg, 1.13 mmol) was dissolved in anhydrous  $\text{CH}_2\text{Cl}_2$  (18 mL) and cooled to 0 °C in an ice bath. DMAP (138 mg, 1.13 mmol) and  $\text{Et}_3\text{N}$  (630 mL, 4.5 mmol) were added before benzoyl chloride (0.197 mL, 1.7 mmol) was added dropwise over 40 minutes. When the addition of benzoyl chloride came to an end the reaction was also done. The reaction mixture was diluted with  $\text{CH}_2\text{Cl}_2$  (5 mL) and successively washed with a solution of sat. aq.  $\text{NaHCO}_3$  (40 mL) and water (30 mL). The organic phase was dried ( $\text{Na}_2\text{SO}_4$ ), filtered and concentrated to give 1 g of a yellow oil that was concentrated on silica and subjected to automated flash chromatography (heptane-EtOAc, 5%  $\rightarrow$  10% EtOAc). The title compound was obtained as a colorless oil (551 mg) in 74% yield.

$R_f$  0.79 (10:1 toluene-EtOAc);  $[\alpha]_D^{20}$  - 79.9 ( $c$  1.0  $\text{CHCl}_3$ ).  $^1\text{H NMR}$  (500 MHz,  $\text{CDCl}_3$ )  $\delta$  8.07 (d,  $J$  = 7.9 Hz, 2H), 7.69 (d,  $J$  = 7.9 Hz, 4H), 7.61 (t,  $J$  = 7.4 Hz, 1H), 7.58 - 7.52 (m, 2H), 7.48 (t,  $J$  = 7.7 Hz, 2H), 7.44 - 7.38 (m, 2H), 7.38 - 7.29 (m, 7H), 5.64 (d,  $J$  = 1.9 Hz, 1H, H-1), 5.59 (dd,  $J$  = 4.6, 1.9 Hz, 1H, H-3), 5.49 (t,  $J$  = 1.9 Hz, 1H, H-2), 4.55 (q,  $J$  = 4.6 Hz, 1H, H-4), 4.06 - 3.95 (m, 4H, H-5a+b,  $\text{CH}_2\text{Cl}$ ), 1.06 (s, 9H, C-( $\text{CH}_3$ )<sub>3</sub>) ppm.  $^{13}\text{C NMR}$  (101 MHz,  $\text{CDCl}_3$ )  $\delta$  166.4, 165.7, 135.8, 133.7, 133.6, 133.3, 133.1, 132.5, 130.1, 129.9, 129.9, 129.2, 129.2, 128.7, 128.4, 128.0, 127.9, 127.8, 90.7 (C-1), 83.4 (C-2), 83.2 (C-4), 77.4 (C-3), 63.4 (C-5), 40.6 ( $\text{CH}_2\text{Cl}$ ), 26.9 (C-( $\text{CH}_3$ )<sub>3</sub>), 19.5 (C-( $\text{CH}_3$ )<sub>3</sub>) ppm. **ESIMS**:  $m/z$  calcd for  $[\text{C}_{36}\text{H}_{37}\text{ClNaO}_6\text{SSi}]^+$ : 683.1661  $[\text{M}+\text{Na}]^+$ , found: 681.1660.

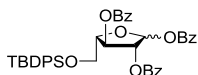
**(101) Phenyl 5-*O*-*tert*-butyldiphenylsilyl-2,3-di-*O*-chloroacetyl-1-thio- $\alpha$ -L-arabinofuranoside**



Compound **101** was obtained as a byproduct in the synthesis of compound **126** as a colorless oil.

**R<sub>f</sub>** 0.55 (2:1 heptane-EtOAc);  $[\alpha]_{\text{D}}^{20}$  - 97.3 (c 1.1 CHCl<sub>3</sub>). **<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>)  $\delta$  7.70 - 7.63 (m, 4H), 7.51 (dd,  $J$  = 6.4, 3.1 Hz, 2H), 7.45 - 7.40 (m, 2H), 7.40 - 7.34 (m, 4H), 7.31 (dd,  $J$  = 5.0, 1.8 Hz, 3H), 5.58 (d,  $J$  = 2.8 Hz, 1H, H-1), 5.45 (dd,  $J$  = 5.5, 2.8 Hz, 1H, H-3), 5.34 (t,  $J$  = 2.8 Hz, 1H, H-2), 4.40 - 4.36 (m, 1H, H-4), 4.11, 4.07 (AB spin,  $J_{\text{AB}}$  = 15.3 Hz, 2H, CH<sub>2</sub>Cl), 4.01 (s, 2H, CH<sub>2</sub>Cl), 3.92 (d,  $J$  = 3.7 Hz, 2H, H-5a+b), 1.06 (s, 9H, C-(CH<sub>3</sub>)<sub>3</sub>) ppm. **<sup>13</sup>C NMR** (101 MHz, CDCl<sub>3</sub>)  $\delta$  166.8, 166.5, 135.8, 133.1, 133.0, 132.5, 130.0, 130.0, 129.2, 128.1, 127.9, 127.9, 90.1 (C-1), 83.0 (C-3), 82.3 (C-4), 78.4 (C-2), 63.0 (C-5), 40.7 (CH<sub>2</sub>Cl), 40.5 (CH<sub>2</sub>Cl), 26.9 (C-(CH<sub>3</sub>)<sub>3</sub>), 19.4 (C-(CH<sub>3</sub>)<sub>3</sub>) ppm. **IR** (neat, cm<sup>-1</sup>): 3071, 2956, 2931, 2857, 1766, 1427, 1282, 1151, 1111, 1024, 740, 701, 606, 503. **ESIMS**:  $m/z$  calcd for [C<sub>31</sub>H<sub>34</sub>Cl<sub>2</sub>NaO<sub>6</sub>SSi]<sup>+</sup>: 655.1115 [M+Na]<sup>+</sup>, found: 655.1114.

#### (102) 1,2,3-Tri-*O*-benzoyl-5-*O*-*tert*-butyldiphenylsilyl-L-arabinofuranose

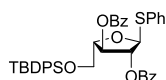


L-Arabinose (50.0 g, 333 mmol) was suspended in anhydrous pyridine (400 mL). 4-(Dimethylamino)pyridine (DMAP) (4.07 g, 33.3 mmol) was added to the reaction mixture. A solution of TBDPSCl (87 mL, 333 mmol) in anhydrous pyridine (125 mL) was added dropwise to the reaction mixture. TLC (10:1 CH<sub>2</sub>Cl<sub>2</sub>-MeOH) indicated that the reaction came to completion after 20 h. The reaction mixture was cooled to 0 °C in an ice bath and benzoyl chloride (124 mL, 1.06 mol) was added. The reaction was stirred for two hours at 23 °C. Afterwards the mixture was diluted with CH<sub>2</sub>Cl<sub>2</sub> (500 mL) and washed with water (3 x 200 mL), 1 M HCl (3 x 200 mL), and a solution of sat. aq. NaHCO<sub>3</sub> (20 mL). The organic phase was dried (MgSO<sub>4</sub>) and the solvent was evaporated under reduced pressure. The resulting oil was dissolved in EtOH (500 mL), from which compound  $\alpha$ -**102** (65 g, 28%) precipitated over 16 hours. The mother liquid was concentrated to a colorless oil that was purified on silica gel (20:1→5:1 heptane-EtOAc) to give compound **102** as a mixture of the  $\alpha$ - and  $\beta$ -anomers as a fluffy solid (88 g, 38%). Total yield over two steps: 66%.

Data for compound  $\alpha$ -**102**: **R<sub>f</sub>** 0.23 (6:1 heptane-EtOAc);  $[\alpha]_{\text{D}}^{20}$  - 5.1 (c 2.1 CHCl<sub>3</sub>); **mp.** 136.3 - 136.5 °C. **<sup>1</sup>H NMR** (300 MHz, CDCl<sub>3</sub>)  $\delta$  8.09-7.99 (m, 4H), 7.94 - 7.83 (m, 2H), 7.71 - 7.15 (m, 19H), 6.58 (s, 1H, H-1), 5.76 (d,  $J$  = 4.5, 1H, H-3), 5.74 (s, 1H, H-2), 4.63 (q,  $J$  = 4.5 Hz, 1H, H-4), 4.06 (dd,

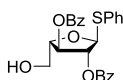
$J = 11.1, 4.5$  Hz, 1H, H-5a), 4.00 (dd,  $J = 11.1, 4.5$  Hz, 1H, H-5b), 1.04 (s, 9H, C-(CH<sub>3</sub>)<sub>3</sub>) ppm. **<sup>13</sup>C NMR** (75 MHz, CDCl<sub>3</sub>)  $\delta$  165.6, 165.3, 165.0, 135.9, 135.8, 133.8, 133.2, 130.2, 130.2, 130.0, 129.7, 129.5, 129.0, 128.7, 128.7, 128.0, 100.2 (C-1), 86.0 (C-4), 81.3 (C-2), 77.3 (C-3), 63.6 (C-5), 27.0 (C-(CH<sub>3</sub>)<sub>3</sub>), 19.5 (C-(CH<sub>3</sub>)<sub>3</sub>) ppm. **IR** (neat, cm<sup>-1</sup>): 3070, 2931, 2858, 1725, 1452, 1258, 1107, 1067, 1024, 705. **ESIMS**:  $m/z$  calcd for [C<sub>42</sub>H<sub>40</sub>NaO<sub>8</sub>Si]<sup>+</sup>: 723.2385 [M+Na]<sup>+</sup>, found: 723.2380.

**(103) Phenyl 2,3-di-*O*-benzoyl-5-*O*-*tert*-butyldiphenylsilyl-1-thio- $\alpha$ -L-arabinofuranoside**



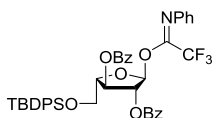
Compound **102** (50 g, 71.3 mmol) was dissolved in anhydrous CH<sub>2</sub>Cl<sub>2</sub> (250 mL) and cooled in a – 78 °C bath. PhSH (10.2 mL, 100 mmol) was added to the stirred mixture along with TMSOTf (5.17 mL, 28.6 mmol). The reaction was allowed to slowly heat to – 20 °C over 16 hours. The mixture was diluted with 20 mL CH<sub>2</sub>Cl<sub>2</sub> and washed with a solution of sat. aq. NaHCO<sub>3</sub> (500 mL) and water (500 mL). The organic phase was dried (MgSO<sub>4</sub>) and concentrated to dryness. The resulting oil was purified on silica gel (40:1→2:1 heptane-EtOAc) to afford compound **103** as a colorless oil (35.9 g, 73%). The analytical data is in accordance with reported data for compound **D-103** ( $R_f$  0.45 (40:1, petroleum–EtOAc);  $[\alpha]_D^{20} + 45.1$  ( $c$  2.0, CHCl<sub>3</sub>)).<sup>203</sup>

$R_f$  0.23 (10:1 heptane-EtOAc),  $[\alpha]_D^{20} - 33.9$  ( $c$  2.0 CHCl<sub>3</sub>). **<sup>1</sup>H NMR** (300 MHz, CDCl<sub>3</sub>)  $\delta$  8.15 - 8.05 (m, 2H), 8.00 - 7.93 (m, 2H), 7.68 (dd,  $J = 7.7, 1.6$  Hz, 4H), 7.65 - 7.26 (m, 17H), 5.76 (d,  $J = 2.0$  Hz, 1H, H-1), 5.71 (dd,  $J = 4.6, 2.0$  Hz, 1H, H-3), 5.65 (t,  $J = 2.0$  Hz, 1H, H-2), 4.62 (q,  $J = 4.6$  Hz, 1H, H-4), 4.02 (d,  $J = 4.6$  Hz, 2H, H-5a+b), 1.05 (s, 9H, C-(CH<sub>3</sub>)<sub>3</sub>) ppm. **<sup>13</sup>C NMR** (75 MHz, CDCl<sub>3</sub>)  $\delta$  165.6, 165.5, 135.8, 135.8, 133.6, 133.3, 132.2, 133.2, 130.1, 130.1, 129.9, 129.8, 129.2, 129.1, 128.6, 128.6, 127.8, 127.7, 91.2 (C-1), 83.4 (C-4), 82.6 (C-2), 77.7 (C-3), 63.6 (C-5), 26.9 (C-(CH<sub>3</sub>)<sub>3</sub>), 19.4 (C-(CH<sub>3</sub>)<sub>3</sub>) ppm. **IR** (neat, cm<sup>-1</sup>): 3070, 2958, 2930, 2857, 1724, 1262, 1107, 705. **ESIMS**:  $m/z$  calcd for [C<sub>41</sub>H<sub>40</sub>NaO<sub>6</sub>SSi]<sup>+</sup>: 711.2207 [M+Na]<sup>+</sup>, found: 711.2202.

**(104) Phenyl 2,3-di-*O*-benzoyl-1-thio- $\alpha$ -L-arabinofuranoside**

A solution of compound **103** (300 mg, 0.435 mmol) in anhydrous THF (3 mL) was cooled to 0 °C in an ice bath. Acetic acid (29.9  $\mu$ L, 0.523 mmol) and TBAF (1 M in THF) (0.435 mL, 0.435 mmol) were added to the reaction mixture. The reaction was stirred for 24 h at 4 °C. After completion of the reaction, the mixture was diluted with 0.5 mL  $\text{CH}_2\text{Cl}_2$  and washed with a solution of sat. aq.  $\text{NH}_4\text{Cl}$ . The organic phase was dried ( $\text{MgSO}_4$ ) and concentrated to dryness. The resulting yellow oil was purified on silica gel (4:1 heptane-EtOAc) to give compound **104** as a fluffy white solid (300 mg, 75%). The NMR data is in accordance with reported data for compound D-**104** ( $[\alpha]_{\text{D}}^{20} + 101.1$  (c 1.0,  $\text{CHCl}_3$ )).<sup>204</sup>

$R_f$  0.22 (2:1 heptane-EtOAc);  $[\alpha]_{\text{D}}^{20} -63.5$  (c 1.0,  $\text{CHCl}_3$ ); **mp.** 101.8 - 102.9 °C.  **$^1\text{H}$  NMR** (300 MHz,  $\text{CDCl}_3$ )  $\delta$  8.13 (d,  $J = 7.3$  Hz, 2H), 8.05 (d,  $J = 7.3$  Hz, 2H), 7.66 - 7.23 (m, 11H), 5.80 (s, 1H, H-1), 5.74 (br, 1H, H-2), 5.56 (dd,  $J = 4.2, 0.8$  Hz, 1H, H-3), 4.59 (q,  $J = 4.2$  Hz, 1H, H-4), 4.07 - 3.95 (m, 2H, H-5a+b) ppm.  **$^{13}\text{C}$  NMR** (75 MHz,  $\text{CDCl}_3$ )  $\delta$  166.2, 165.5, 134.0, 133.9, 132.4, 130.3, 130.1, 129.3, 129.2, 129.1, 128.8, 128.1, 91.7 (C-1), 84.0 (C-4), 82.4 (C-2), 78.4 (C-3), 62.2 (C-5) ppm. **IR** (neat,  $\text{cm}^{-1}$ ): 3513, 3062, 2929, 1719, 1451, 1249, 1094, 1067, 1025, 707. **ESIMS**:  $m/z$  calcd for  $[\text{C}_{25}\text{H}_{22}\text{NaO}_6\text{S}]^+$ : 473.1029  $[\text{M}+\text{Na}]^+$ , found: 473.1028.

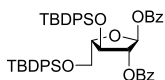
**(105) 2,3-Di-*O*-benzoyl-5-*O*-*tert*-butyldiphenylsilyl- $\alpha$ -L-arabinofuranosyl *N*-phenyl trifluoroacetimidate**

Compound **86** (600 mg, 1.0 mmol) was added to a solution of  $\text{Cs}_2\text{CO}_3$  (655 mg, 2.0 mmol) and trifluoroacetimidyl chloride (0.323 mL, 2.0 mmol) in acetone (9 mL). The reaction came to completion within 1.5 hours, then it was filtered through Celite and concentrated to give 1.03 g of a yellow oil. The mixture was

subjected to automated flash chromatography (heptane-EtOAc, 0% → 25% EtOAc) and the title compound was obtained as a colorless oil (724 mg, 93%).

$R_f$  0.8 (2:1 heptane-EtOAc),  $[\alpha]_D^{20} + 15.4$  ( $c$  0.96  $\text{CHCl}_3$ ).  $^1\text{H NMR}$  (300 MHz,  $\text{CDCl}_3$ )  $\delta$  8.18 - 6.77 (m, 25H), 6.60 - 6.40 (m, 1H), 5.75 - 5.65 (m, 2H), 4.60 (d,  $J = 5.0$  Hz, 1H), 4.01 (d,  $J = 5.0$  Hz, 2H), 1.04 (s, 9H) ppm.  $^{13}\text{C NMR}$  (75 MHz,  $\text{CDCl}_3$ )  $\delta$  135.8, 135.8, 133.8, 133.7, 131.4, 130.1, 130.1, 129.9, 129.6, 128.9, 128.6, 127.9, 120.6, 86.3, 76.7, 71.2, 63.5, 26.9, 19.4 ppm. **ESIMS**:  $m/z$  calcd for  $[\text{C}_{43}\text{H}_{40}\text{F}_3\text{NaO}_7\text{Si}]^+$ : 790.2418, found: 790.2423.

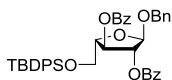
**(106) 1,2-Di-*O*-benzoyl-3,5-di-*O*-*tert*-butyldiphenylsilyl- $\alpha$ -L-arabinofuranose**



Compound **106** was obtained as a clear oil as a byproduct in the synthesis of compound **102**.

$R_f$  0.33 (10:1 heptane-EtOAc);  $[\alpha]_D^{20} + 16.0$  ( $c$  1.7  $\text{CHCl}_3$ );  $^1\text{H NMR}$  (300 MHz,  $\text{CDCl}_3$ )  $\delta$  8.15 - 8.10 (m, 2H), 7.92 - 7.23 (m, 28H), 5.58 (s, 1H, H-2), 5.57 (s, 1H, H-1), 5.51 (d,  $J = 4.3$  Hz, 1H, H-3), 4.60 (q,  $J = 4.3$ , 1H, H-4), 4.00 - 3.96 (m, 2H, H-5a+b), 1.13 (s, 9H,  $\text{C}-(\text{CH}_3)_3$ ), 1.02 (s, 9H,  $\text{C}-(\text{CH}_3)_3$ ) ppm,  $^{13}\text{C NMR}$  (75 MHz,  $\text{CDCl}_3$ )  $\delta$  165.8, 165.3, 135.9, 135.9, 135.8, 133.5, 133.3, 133.2, 133.1, 130.1, 130.0, 129.9, 129.8, 129.8, 129.6, 128.5, 128.4, 127.8, 127.8, 127.8, 127.8, 101.3 (C-1), 84.3 (C-4), 83.1 (C-2), 77.9 (C-3), 64.3 (C-5), 26.9 ( $2 \times \text{C}-(\text{CH}_3)_3$ ), 19.6 ( $\text{C}-(\text{CH}_3)_3$ ), 19.4 ( $\text{C}-(\text{CH}_3)_3$ ) ppm. **IR** (neat,  $\text{cm}^{-1}$ ): 3071, 2958, 2931, 2857, 1722, 1427, 1260, 1105, 1057, 1027, 699, 504. **ESIMS**:  $m/z$  calcd for  $[\text{C}_{51}\text{H}_{54}\text{NaO}_7\text{S}_2]^+$ : 857.3300  $[\text{M}+\text{Na}]^+$ , found: 857.3294.

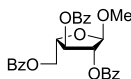
**(107) Benzyl 2,3-di-*O*-benzoyl-5-*O*-*tert*-butyldiphenylsilyl- $\alpha$ -L-arabinofuranoside**



To a solution of compound **102** (10.73 g, 15.35 mmol) in anhydrous  $\text{CH}_2\text{Cl}_2$  (80 mL) was added activated and partly powdered molecular sieves (7.5 g) and  $\text{BnOH}$  (9.75 mL, 94.2 mmol). The mixture was stirred for 45 minutes before boron trifluoride diethyl etherate (5.57 mL, 47 mmol) was added over 45 minutes. TLC showed completion after 3 hours. The reaction was cooled to 0 °C in an ice bath before it was quenched with  $\text{Et}_3\text{N}$  (11 mL). The mixture was concentrated and purified by column chromatography (10:1 heptane-EtOAc) to afford compound **107** as a colorless oil (9.38 g, 89%).

$R_f$  0.49 (2:1 heptane-EtOAc),  $[\alpha]_D^{20}$  -2.7 ( $c$  1.0,  $\text{CHCl}_3$ ).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  8.07 - 8.02 (m, 2H), 8.00 - 7.94 (m, 2H), 7.76 - 7.69 (m, 4H), 7.62 - 7.52 (m, 2H), 7.47 - 7.27 (m, 15H), 5.62 (dd,  $J$  = 4.8, 1.0 Hz, 1H, H-3), 5.54 (d,  $J$  = 1.0 Hz, 1H, H-2), 5.32 (s, 1H, H-1), 4.86 (d,  $J$  = 11.9 Hz, 1H,  $\text{CH}_2\text{Ph}$ ), 4.63 (d,  $J$  = 11.9 Hz, 1H,  $\text{CH}_2\text{Ph}$ ), 4.43 (q,  $J$  = 4.8 Hz, 1H, H-4), 4.03 (d,  $J$  = 4.8 Hz, 2H, H-5a+b), 1.06 (s, 9H,  $\text{C}-(\text{CH}_3)_3$ ) ppm.  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  165.7, 165.5, 159.2, 137.6, 136.6, 135.8, 134.9, 133.5, 133.4, 133.4, 130.1, 130.1, 129.8, 129.6, 129.3, 128.5, 128.5, 128.0, 127.8, 105.0 (C-1), 83.4 (C-4), 82.4 (C-2), 77.6 (C-3), 68.7 ( $\text{CH}_2\text{Ph}$ ), 63.8 (C-5), 26.9 ( $\text{C}-(\text{CH}_3)_3$ ), 19.4 ( $\text{C}-(\text{CH}_3)_3$ ) ppm. ESIMS:  $m/z$  calcd for  $[\text{C}_{42}\text{H}_{42}\text{NaO}_7\text{Si}]^+$ : 709.2592  $[\text{M}+\text{Na}]^+$ , found: 709.2585.

#### (108) Methyl 2,3,5-tri-*O*-benzoyl- $\alpha$ -L-arabinofuranoside<sup>205</sup>

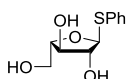


The title compound **108** was synthesized by the procedure described for the synthesis of the D-enantiomer and obtained in 38% yield as white crystals.<sup>206</sup> The analytic data is in accordance with literature values ( $R_f$  0.75 (3:1 petroleum ether-EtOAc); mp. 106 °C).<sup>205</sup>

$R_f$  0.18 (5:1 heptane-EtOAc);  $[\alpha]_D^{20}$  +20.5 ( $c$  2.0  $\text{CHCl}_3$ ); mp. 99.7 - 100.7 °C.  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  8.12 - 7.97 (m, 6H), 7.66 - 7.22 (m, 9H), 5.59 (dd,  $J$  = 5.2, 1.3 Hz, 1H, H-3), 5.52 (d,  $J$  = 1.3 Hz, 1H, H-2), 5.19 (s, 1H, H-1), 4.85 (dd,  $J$  = 11.9, 3.4 Hz, 1H, H-5a), 4.69 (dd,  $J$  = 11.9, 4.8 Hz, 1H, H-5b), 4.60 - 4.56 (m, 1H, H-4), 3.50 (s, 3H,  $\text{CH}_3$ ) ppm.  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  166.4, 166.1, 165.7, 133.8, 133.7, 133.3, 130.2, 130.1, 130.0, 129.9, 129.3, 129.3, 128.7, 128.7, 128.5, 107.1 (C-1), 82.4 (C-2), 81.1 (C-4),

78.2 (C-3), 63.9 (C-5), 55.3 ( $\underline{\text{C}}\text{H}_3$ ) ppm. **IR** (neat,  $\text{cm}^{-1}$ ): 2936, 1712, 1600, 1452, 1255, 1109, 707.

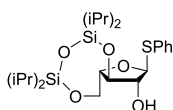
**(109) Phenyl 1-thio- $\alpha$ -L-arabinofuranoside**<sup>170</sup>



By using the synthetic procedure described for the D-enantiomer,<sup>203</sup> the title compound **109** was obtained in 89% yield as a colorless oil. Analytical data for the compound dissolved in methanol is reported in literature ( $R_f$  0.5 (9:1  $\text{CH}_2\text{Cl}_2$ -MeOH);  $[\alpha]_D^{20} - 145.2$  (c 0.25 MeOH)).<sup>170</sup>

$R_f$  0.24 (20:1  $\text{CH}_2\text{Cl}_2$ -MeOH);  $[\alpha]_D^{20} - 186$  (c 2.0  $\text{CHCl}_3$ ).  **$^1\text{H}$  NMR** (300 MHz,  $\text{CDCl}_3$ )  $\delta$  7.50 - 7.44 (m, 2H), 7.32 - 7.23 (m, 3H), 5.42 (d,  $J = 3.2$  Hz, 1H, H-1), 4.18 - 4.10 (m, 3H, H-2, H-3, H-4), 3.84 (dd,  $J = 12.6, 2.0$  Hz, 1H, H-5a), 3.72 (dd,  $J = 12.6, 2.0$  Hz, 1H, H-5b) ppm.  **$^{13}\text{C}$  NMR** (100 MHz,  $\text{CDCl}_3$ )  $\delta$  133.7, 131.9, 129.1, 127.7, 91.9 (C-1), 83.4 (C-2), 81.9 (C-4), 76.9 (C-3), 61.1 (C-5) ppm. **IR** (neat,  $\text{cm}^{-1}$ ): 3347, 3060, 2924, 1439, 1023, 740.

**(110) Phenyl 3,5-O-(1,1,3,3-tetraisopropylidisiloxane-1,3-diyl)-1-thio- $\alpha$ -L-arabinofuranoside**<sup>150</sup>

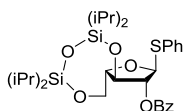


Compound **109** (9.8 g, 40.5 mmol) was dissolved in pyridine (100 mL) and cooled to 0 °C in an ice bath. TIPDSCl<sub>2</sub> (15.6 g, 49.5 mmol) and DMAP (200 mg, 1.64 mmol) were added and the reaction mixture was slowly heated to 22 °C under which the color changed from clear brown to milky brown. TLC indicated that the reaction came to completion after 2 hours. The mixture was diluted with  $\text{CH}_2\text{Cl}_2$  (100 mL) and washed with water (2 x 100 mL) and solution of sat. aq.  $\text{NaHCO}_3$  (100 mL). The organic phase was dried ( $\text{MgSO}_4$ ) and concentrated to give a yellow oil (24 g) that was purified on a flash column (20:1 heptane-EtOAc) to get 16.1 g colorless oil (82% yield). Only HRMS data is reported in the literature.<sup>150</sup>



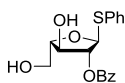
$R_f$  0.32 (6:1 heptane-EtOAc);  $[\alpha]_D^{20}$  - 80.9 (*c* 1.3  $\text{CHCl}_3$ ).  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  7.53 - 7.51 (m, 1H), 7.51 - 7.48 (m, 1H), 7.32 - 7.23 (m, 4H), 5.33 (d,  $J$  = 5.5 Hz, 1H, H-1), 4.27 - 4.19 (m, 2H, H-2, H-4), 4.00 (d,  $J$  = 3.3 Hz, 2H, H-5a+b), 3.98 - 3.93 (m, 1H, H-3), 2.27 (d,  $J$  = 4.5 Hz, 1H, OH), 1.12 - 1.02 (m, 28H, *i*-Pr) ppm.  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  134.9, 131.3, 129.1, 127.4, 91.0 (C-1), 82.1 (C-4), 80.7 (C-2), 76.2 (C-3), 61.3 (C-5), 17.6, 17.5 (2C), 17.5, 17.3, 17.2 (2C), 17.1, 13.7, 13.3, 12.9, 12.7 ppm. IR (neat,  $\text{cm}^{-1}$ ): 3459, 2944, 2894, 2867, 1464, 1152, 1082, 1033, 883, 858, 689.

**(111) Phenyl 2-*O*-benzoyl-3,5-*O*-(1,1,3,3-tetraisopropylidisiloxane-1,3-diyl)-1-thio- $\alpha$ -L-arabinofuranoside**



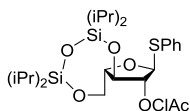
Compound **110** (6.5 g, 13.4 mmol) was dissolved in pyridine (50 mL). Benzoyl chloride (3.43 mL, 29.5 mmol) was added dropwise and the reaction came to an end after one hour. Afterwards it was quenched by the addition of water (100 mL). The phases were separated and the organic phase was washed with brine (100 mL), dried ( $\text{MgSO}_4$ ), and concentrated. After column chromatography (20:1 heptane-EtOAc) 7.0 g of the title product was obtained as a colorless oil (89% yield). The NMR data is in accordance with reported data for compound D-**111** ( $R_f$  0.40 (100:1 petroleum ether-EtOAc),  $[\alpha]_D^{20}$  + 48.4 (*c* 2.00,  $\text{CHCl}_3$ )).<sup>178</sup>

$R_f$  0.11 (20:1 heptane-EtOAc);  $[\alpha]_D^{20}$  - 24.3 (*c* 0.91  $\text{CHCl}_3$ ).  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  8.07 - 8.02 (m, 2H), 7.64 - 7.39 (m, 5H), 7.33 - 7.17 (m, 3H), 5.60 (dd,  $J$  = 5.3, 3.7 Hz, 1H, H-2), 5.55 (d,  $J$  = 3.7 Hz, 1H, H-1), 4.58 (dd,  $J$  = 7.8, 5.3 Hz, 1H, H-3), 4.23 (dt,  $J$  = 7.8, 3.7 Hz, 1H, H-4), 4.10 (dd,  $J$  = 12.7, 3.7, 1H, H-5a), 4.03 (dd,  $J$  = 12.7, 3.7, 1H, H-5b), 1.21 - 0.79 (m, 28H, *i*-Pr) ppm.  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  165.6, 134.7, 133.5, 131.5, 129.9, 129.5, 129.0, 128.6, 127.4, 89.5 (C-1), 83.3 (C-2), 81.0 (C-4), 75.5 (C-3), 61.4 (C-5), 17.6, 17.5 (3C), 17.1, 17.1 (2C), 17.0, 13.6, 13.3, 13.0, 12.6 ppm. IR (neat,  $\text{cm}^{-1}$ ): 2944, 2867, 1732, 1464, 1387, 1265, 1108, 1082, 1054, 1038, 992, 883, 688. ESIMS:  $m/z$  calcd for  $[\text{C}_{30}\text{H}_{44}\text{NaO}_6\text{SSi}_2]^+$ : 611.2289  $[\text{M}+\text{Na}]^+$ , found: 611.2282.

**(112) Phenyl 2-*O*-benzoyl-1-thio- $\alpha$ -L-arabinofuranoside**

A solution of compound **111** (2.9 g, 4.92 mmol) and AcOH (0.564 mL, 2 equiv.) in anhydrous THF (29 mL) under Ar was cooled to 0 °C in an ice bath. TBAF (1 M in THF) (4.92 mL, 4.92 mmol) was added and the reaction was allowed to heat to 20 °C. The reaction was done after two hours and the reaction mixture was successively washed with a solution of sat. aq. (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> (50 mL) and brine (50 mL). The organic phase was dried (MgSO<sub>4</sub>) and concentrated before column chromatographed (4:1 heptane-EtOAc) to yield 72% of the title product as a colorless oil. The analytical data is in accordance with data reported for the D-enantiomer (*R<sub>f</sub>* 0.35 (1:1 petroleum-EtOAc); [ $\alpha$ ]<sub>D</sub><sup>20</sup> + 173.5 (*c* 1.42 CHCl<sub>3</sub>)).<sup>203</sup>

*R<sub>f</sub>* 0.15 (2:1 heptane-EtOAc); [ $\alpha$ ]<sub>D</sub><sup>20</sup> - 174.3 (*c* 0.92 CHCl<sub>3</sub>). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  8.06 - 8.00 (m, 2H), 7.65 - 7.52 (m, 3H), 7.51 - 7.41 (m, 2H), 7.39 - 7.29 (m, 3H), 5.76 (d, *J* = 3.3 Hz, 1H, H-1), 5.15 (t, *J* = 3.3 Hz, 1H, H-2), 4.40 - 4.34 (m, 1H, H-3), 4.34 - 4.27 (m, 1H, H-4), 3.98 (dd, *J* = 12.3, 3.0 Hz, 1H, H-5a), 3.82 (dd, *J* = 12.3, 3.8 Hz, 1H, H-5b) ppm. <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$  167.5 134.0, 133.7, 132.2, 130.1, 129.2, 128.9, 128.8, 128.0, 127.7, 89.5 (C-1), 87.7 (C-2), 82.8 (C-3), 76.4 (C-4), 61.4 (C-5) ppm. IR (neat, cm<sup>-1</sup>): 3401, 3061, 2927, 2873, 1707, 1266, 1110, 1068, 1024, 986, 742, 709, 688. ESIMS: *m/z* calcd for [C<sub>18</sub>H<sub>18</sub>NaO<sub>5</sub>S]<sup>+</sup>: 369.0767 [M+Na]<sup>+</sup>, found: 369.0769.

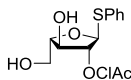
**(113) Phenyl 2-*O*-chloroacetyl-3,5-*O*-(1,1,3,3-tetraisopropylidisiloxane-1,3-diyl)-1-thio- $\alpha$ -L-arabinofuranoside**

Alcohol **110** (5.0 g, 10.3 mmol) was dissolved in anhydrous pyridine (100 mL). Chloroacetic anhydride (2.4 g, 14.0 mmol) was added and the reaction was done after 15 minutes. Most of the solvent was evaporated and the remaining mixture was successively washed with a solution of sat. aq. NaHCO<sub>3</sub> (2 x 150 mL) and water (150 mL). The organic phase was dried (MgSO<sub>4</sub>),

concentrated, and purified by flash chromatography (20:1 heptane-EtOAc). The title product was obtained in 78% yield as a colorless oil.

**R<sub>f</sub>** 0.22 (20:1 heptane-EtOAc),  $[\alpha]_D^{20}$  -49.6 (*c* 0.77 CHCl<sub>3</sub>). **<sup>1</sup>H NMR** (300 MHz, CDCl<sub>3</sub>)  $\delta$  7.58 - 7.46 (m, 2H), 7.34 - 7.19 (m, 3H), 5.41 (d, *J* = 1.3 Hz, 1H, H-2), 5.40 (s, 1H, H-1), 4.45 - 4.38 (m, 1H, H-3), 4.17 - 4.10 (m, 1H, H-4), 4.08 (d, *J* = 1.3 Hz, 2H, CH<sub>2</sub>Cl), 4.04 (dd, *J* = 12.7, 3.1 Hz, 1H, H-5a), 3.97 (dd, *J* = 12.7, 4.4, 1H, H-5b), 1.16 - 0.91 (m, 28H, *i*-Pr) ppm. **<sup>13</sup>C NMR** (75 MHz, CDCl<sub>3</sub>)  $\delta$  166.4, 134.2, 131.8, 129.0, 127.6, 88.8 (C-1), 84.3 (C-2), 81.1 (C-4), 75.2 (C-3), 61.3 (C-5), 40.7 (CH<sub>2</sub>Cl), 17.6, 17.4, 17.3, 17.2, 17.1, 17.0, 13.6, 13.3, 13.3, 12.9, 12.8, 12.6 ppm. **IR** (neat, cm<sup>-1</sup>): 2944, 2867, 1773, 1754, 1464, 1387, 1249, 1147, 1108, 1081, 1035, 991, 883, 852, 689. **ESIMS**: *m/z* calcd for [C<sub>25</sub>H<sub>41</sub>ClNaO<sub>6</sub>SSi<sub>2</sub>]<sup>+</sup>: 583.1743 [M+Na]<sup>+</sup>, found: 583.1738.

#### (114) Phenyl 2-*O*-chloroacetyl-1-thio- $\alpha$ -L-arabinofuranoside

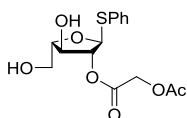


Compound **113** (400 mg, 0.71 mmol) was dried under vacuum and dissolved in anhydrous THF (4 mL). The reaction was cooled to 0 °C in an ice bath. Acetic Acid (0.163 mL, 2.85 mmol) and TBAF (1M in THF) (1.43 mL, 1.43 mmol) were added. The reaction was stirred for 40 minutes at which point TCL showed complete conversion of the starting material. The reaction mixture was diluted with 1 mL CH<sub>2</sub>Cl<sub>2</sub>, washed with a solution of sat. aq. (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> (5 mL), brine (5 mL), and water (5 mL). The organic phase was dried (MgSO<sub>4</sub>), filtered, and concentrated to a yellow oil that was subjected to automated flash chromatography (heptane-EtOAc, 50% → 60% EtOAc) to afford 100 mg title product as white crystals (44% yield).

**R<sub>f</sub>** 0.10 (2:1 heptane-EtOAc);  $[\alpha]_D^{20}$  -197.2 (*c* 0.78 CHCl<sub>3</sub>); **mp.** 89.9 - 93.0 °C. **<sup>1</sup>H NMR** (300 MHz, CDCl<sub>3</sub>)  $\delta$  7.54 - 7.49 (m, 2H), 7.37 - 7.29 (m, 3H), 5.58 (d, *J* = 3.1 Hz, 1H, H-1), 5.05 (t, *J* = 3.1 Hz, 1H, H-2), 4.32 - 4.26 (m, 1H, H-4), 4.23 (ddd, *J* = 7.2, 3.1, 0.6 Hz, 1H, H-3), 4.13 (s, 2H, CH<sub>2</sub>Cl), 3.93 (dd, *J* = 12.4, 2.9 Hz, 1H, H-5a), 3.78 (dd, *J* = 12.4, 3.4 Hz, 1H, H-5b) ppm. **<sup>13</sup>C NMR** (75 MHz, CDCl<sub>3</sub>)  $\delta$  168.1, 133.2, 132.4, 129.3, 128.2, 89.3 (C-1), 87.9 (C-2), 83.0 (C-4), 76.0 (C-3), 61.1 (C-5), 40.7 (CH<sub>2</sub>Cl) ppm. **IR** (neat, cm<sup>-1</sup>): 3443, 3268, 2959, 2933, 2890, 2868, 1755, 1391, 1320, 1181, 1091,

1037, 1001, 978, 870, 775, 753, 693. **ESIMS**:  $m/z$  calcd for  $[C_{13}H_{15}ClNaO_5S]^+$ : 341.0221  $[M+Na]^+$ , found: 341.0222.

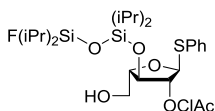
**(115) Phenyl 2-*O*-acetoxyacetyl-1-thio- $\alpha$ -L-arabinofuranoside**



Compound **115** was obtained as a colorless oil as a byproduct in the synthesis of compound **114**.

$R_f$  0.05 (1:1 heptane-EtOAc);  $[\alpha]_D^{20}$  - 184.9 ( $c$  1.0  $CHCl_3$ ).  **$^1H$  NMR** (300 MHz,  $CDCl_3$ )  $\delta$  7.55 - 7.46 (m, 2H), 7.38 - 7.27 (m, 3H), 5.56 (d,  $J$  = 3. Hz, 1H, H-1), 5.03 (t,  $J$  = 3.0 Hz, 1H, H-2), 4.65 (s, 2H,  $CH_2$ ), 4.34 - 4.24 (m, 1H, H-4), 4.23 - 4.18 (m, 1H, H-3), 3.93 (dd,  $J$  = 12.4, 2.9 Hz, 1H, H-5a), 3.78 (dd,  $J$  = 12.4, 3.6 Hz, 1H, H-5b), 2.17 (s, 3H,  $CH_3$ ) ppm.  **$^{13}C$  NMR** (101 MHz,  $CDCl_3$ )  $\delta$  170.6, 168.6, 133.3, 132.4, 129.2, 128.1, 89.3 (C-1), 87.4 (C-2), 82.9 (C-4), 76.0 (C-3), 61.2 (C-5), 60.7 ( $CH_2$ ), 20.5 ( $CH_3$ ). **IR** (neat,  $cm^{-1}$ ): 3433, 2935, 1743, 1389, 1372, 1277, 1186, 1078, 1024, 740, 691. **ESIMS**:  $m/z$  calcd for  $[C_{15}H_{18}NaO_7S]^+$ : 365.0665  $[M+Na]^+$ , found: 365.0658.

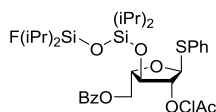
**(116) Phenyl 2-*O*-chloroacetyl-3-*O*-(1-fluoro-1,1,3,3-tetraisopropyl-disiloxane-1,3-diyl)-1-thio- $\alpha$ -L-arabinofuranoside**



A solution of compound **113** (100 mg, 0.178 mmol) in  $CH_2Cl_2/MeCN$  (3.9 mL:11.7 mL) was cooled to 0 °C in an ice bath before HF (40% in water, 0.700 mL) was added dropwise. The reaction was stirred at 0 °C for 1 hour. Afterwards it was quenched with  $MeOSiMe_3$  (2.6 mL) and stirred for another 40 minutes.  $NaHCO_3$  (22 mg) was added and the mixture was concentrated on vacuum. After flash chromatography (10:1 heptane-EtOAc), 75 mg of the title compound was obtained as a colorless oil (75%).

$R_f$  0.41 (2:1 heptane-EtOAc);  $[\alpha]_D^{20}$  - 83.8 ( $c$  0.93  $\text{CHCl}_3$ ).  $^1\text{H NMR}$  (300 MHz,  $\text{CDCl}_3$ )  $\delta$  7.52 (dt,  $J = 4.3, 2.4$  Hz, 2H), 7.37 - 7.23 (m, 3H), 5.50 (br, 1H, H-1), 5.24 (t,  $J = 1.9$  Hz, 1H, H-2), 4.52 (dd,  $J = 4.9, 1.9$  Hz, 1H, H-3), 4.36 (dt,  $J = 4.9, 3.3$  Hz, 1H, H-4), 4.06 (s, 2H,  $\text{CH}_2\text{Cl}$ ), 3.92 (dd,  $J = 12.3, 3.3$  Hz, 1H, H-5a), 3.77 (dd,  $J = 12.1, 3.3$  Hz, 1H, H-5b), 1.14 - 0.91 (m, 28H, *i*-Pr) ppm.  $^{13}\text{C NMR}$  (75 MHz,  $\text{CDCl}_3$ )  $\delta$  166.35, 134.5, 132.2, 129.1, 127.8, 91.3 (C-1), 86.2 (C-2), 85.3 (C-3), 75.4 (C-4), 61.4 (C-5), 40.6 ( $\text{CH}_2\text{Cl}$ ), 17.1, 17.1, 16.8, 16.8, 13.0, 12.7, 12.5. **IR** (neat,  $\text{cm}^{-1}$ ): 2947, 2869, 1768, 1752, 1464, 1161, 1132, 1085, 1055, 883, 837, 690. **ESIMS**:  $m/z$  calcd for  $[\text{C}_{25}\text{H}_{42}\text{ClFNaO}_6\text{SSi}_2]^+$ : 603.1805  $[\text{M}+\text{Na}]^+$ , found: 603.1807.

**(117) Phenyl 5-*O*-benzoyl-2-*O*-chloroacetyl-3-*O*-(1-fluoro-1,1,3,3-tetraiso-propyldisiloxane-1,3-diyl)-1-thio- $\alpha$ -L-arabinofuranoside**

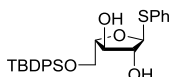


Compound **116** (140 mg, 0.241 mmol) was dissolved in pyridine (1.4 mL) and cooled to 0 °C in an ice bath before benzoyl chloride (61.4  $\mu\text{L}$ , 0.530 mmol) was added. TLC indicated that the reaction had run to completion after four hours, so it was quenched with water (1 mL) and stirred for 20 minutes. The mixture was extracted with  $\text{CH}_2\text{Cl}_2$  (3 x 5 mL). The combined organic phases were successively washed with a solution of sat. aq.  $\text{NaHCO}_3$  (20 mL), 1 M HCl (20 mL), and water (20 mL), dried ( $\text{MgSO}_4$ ) and concentrated to a colorless oil. After flash chromatography (heptane-EtOAc, 1:0  $\rightarrow$  3:1) the title compound was isolated in 83% yield as a colorless oil.

$R_f$  0.68 (2:1 heptane-EtOAc);  $[\alpha]_D^{20}$  - 78.9 ( $c$  0.95  $\text{CHCl}_3$ ).  $^1\text{H NMR}$  (300 MHz,  $\text{CDCl}_3$ )  $\delta$  8.11 - 8.00 (m, 2H), 7.63 - 7.49 (m, 3H), 7.44 (t,  $J = 7.7$  Hz, 2H), 7.33 - 7.22 (m, 3H), 5.54 (s, 1H, H-1), 5.26 (d,  $J = 1.3$  Hz, 1H, H-2), 4.71 - 4.59 (m, 2H, H-3, H-5a), 4.59 - 4.53 (m, 1H, H-4), 4.53 - 4.42 (m, 1H, H-5b), 3.96 (s, 2H,  $\text{CH}_2\text{Cl}$ ), 1.12 - 0.97 (m, 28H, *i*-Pr) ppm.  $^{13}\text{C NMR}$  (75 MHz,  $\text{CDCl}_3$ )  $\delta$  166.5, 166.4, 134.6, 133.5, 132.3, 130.0, 129.2, 128.6, 127.8, 91.6 (C-1), 86.3 (C-2), 83.5 (C-3), 76.4 (C-4), 63.4 (C-5), 40.6 ( $\text{CH}_2\text{Cl}$ ), 17.2, 17.2, 17.2, 16.8, 16.8, 16.8, 16.7, 16.7, 13.1, 13.1, 12.8, 12.6 ppm. **IR** (neat,  $\text{cm}^{-1}$ ): 2947, 2895, 2869, 1770, 1723, 1464, 1269, 1123, 1085, 1057,

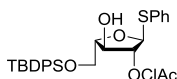
1025, 883, 837, 689. **ESIMS**:  $m/z$  calcd for  $[\text{C}_{32}\text{H}_{46}\text{ClFNaO}_7\text{SSi}_2]^+$ : 707.2067  $[\text{M}+\text{Na}]^+$ , found: 707.2070.

**(125) Phenyl 5-*O*-*tert*-butyldiphenylsilyl-1-thio- $\alpha$ -L-arabinofuranoside**



A solution of compound **109** (7.8 g, 32.2 mmol) in pyridine (90 mL) was cooled to 0 °C in an ice bath. TBDPSCl (13.5 mL, 51.9 mmol) was added dropwise over 20 minutes. The reaction temperature was raised from the ice bath and allowed to heat to room temperature. TLC showed completion after 2 hours, so the reaction mixture was diluted with  $\text{CH}_2\text{Cl}_2$  (100 mL) and washed with a solution of sat. aq.  $\text{NH}_4\text{Cl}$  (100 mL), 0.5 M HCl (100 mL), a solution of sat. aq.  $\text{NaHCO}_3$  (100 mL), and water (100 mL). The organic phase was dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and concentrated to give 24 g of a yellow oil that was purified on a dry column (heptane-EtOAc) to get 15.7 g colorless oil (90% yield). The analytical data is in accordance with reported data for compound **D-125** ( $R_f$  0.3 (2:1 petroleum-EtOAc);  $[\alpha]_{\text{D}}^{20} + 132.7$  (c 1.25  $\text{CHCl}_3$ )).<sup>203</sup>

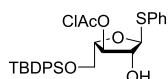
$R_f$  0.44 (1:1 heptane-EtOAc);  $[\alpha]_{\text{D}}^{20} - 141.4$  (c 0.79  $\text{CHCl}_3$ ).  **$^1\text{H}$  NMR** (300 MHz,  $\text{CDCl}_3$ )  $\delta$  8.12 - 7.99 (m, 1H), 7.76 - 7.61 (m, 4H), 7.61 - 7.11 (m, 10H), 5.61 (d,  $J = 1.7$  Hz, 1H, H-1), 4.31 (br, 1H, H-3), 4.26 (q,  $J = 2.6$  Hz, 1H, H-4), 4.22 (br, 1H, H-2), 3.87 (dd,  $J = 11.3, 2.6$  Hz, 1H, H-5a), 3.80 (dd,  $J = 11.3, 2.6$  Hz, 1H, H-5b), 1.12 (s, 9H, C-( $\text{CH}_3$ )<sub>3</sub>) ppm.  **$^{13}\text{C}$  NMR** (75 MHz,  $\text{CDCl}_3$ )  $\delta$  135.9, 135.8, 132.1, 130.4, 130.3, 129.3, 128.2, 128.1, 93.1 (C-1), 86.4 (C-4), 81.4 (C-2), 79.0 (C-3), 64.3 (C-5), 27.0 (C-( $\text{CH}_3$ )<sub>3</sub>), 19.3 (C-( $\text{CH}_3$ )<sub>3</sub>) ppm. **IR** (neat,  $\text{cm}^{-1}$ ): 3386, 3071, 2929, 2857, 1427, 1111, 1061, 1025, 739, 700, 504. **ESIMS**:  $m/z$  calcd for  $[\text{C}_{27}\text{H}_{32}\text{NaO}_4\text{SSi}]^+$ : 503.1683  $[\text{M}+\text{Na}]^+$ , found: 503.1685.

**(126) Phenyl 5-*O*-*tert*-butyldiphenylsilyl-2-*O*-chloroacetyl-1-thio- $\alpha$ -L-arabinofuranoside**

A solution of diol **125** (9 g, 18.7 mmol) and DMAP (230 mg, 1.9 mmol) in  $\text{CH}_2\text{Cl}_2$  (175 mL) was cooled to 0 °C in an ice bath. Chloroacetic anhydride (3.84 g, 22.5 mmol) was dissolved in  $\text{CH}_2\text{Cl}_2$  (50 mL) and added to the reaction mixture at 5 mmol/h. After the addition, the reaction mixture was successively washed with a solution of sat. aq.  $\text{NaHCO}_3$  (200 mL) and water (2 x 200 mL). The organic phase was dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and concentrated to give 10.8 g of a yellow oil that was purified on a dry column in toluene-EtOAc to get the title compound as a colorless oil (3.9 g, 7.0 mmol) in 37% yield. Additionally two monosaccharides were isolated: one where the chloroacetyl protects the three position (**127**) (1.6 g, 2.8 mmol) in 15% and the double chloroacetyl protected (**101**) (3.4 g, 5.4 mmol) in 29%. Further 1.65 g (3.4 mmol, 18% of the starting material (**125**)) was isolated.

Data for compound **126**:  $R_f$  0.47 (10:1 toluene-EtOAc),  $[\alpha]_D^{20}$  -113.4 (*c* 0.93  $\text{CHCl}_3$ ).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.70 - 7.64 (m, 4H), 7.54 - 7.50 (m, 2H), 7.47 - 7.34 (m, 6H), 7.33 - 7.28 (m, 3H), 5.58 (d,  $J$  = 3.2 Hz, 1H, H-1), 5.04 (t,  $J$  = 3.2 Hz, 1H, H-2), 4.32 - 4.25 (m, 2H, H-3, H-4), 4.08, 4.05 (AB spin,  $J_{AB}$  = 15.2 Hz, 2H,  $\text{CH}_2\text{Cl}$ ), 3.92 (dd,  $J$  = 11.3, 3.3 Hz, 1H, H-5a), 3.88 (dd,  $J$  = 11.3, 3.3 Hz, 1H, H-5b), 1.06 (s, 9H, C-( $\text{CH}_3$ )<sub>3</sub>) ppm.  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$  168.1, 135.8, 135.7, 133.5, 133.6, 133.2, 132.3, 131.6, 129.9, 129.2, 128.1, 128.0, 127.9, 127.9, 127.9, 89.0 (C-1), 87.9 (C-2), 83.4 (C-4), 76.8 (C-3), 63.1 (C-5), 40.6 ( $\text{CH}_2\text{Cl}$ ), 26.9 (C-( $\text{CH}_3$ )<sub>3</sub>), 19.4 ( $\text{C}-(\text{CH}_3)_3$ ). IR (neat,  $\text{cm}^{-1}$ ): 2929, 2856, 1749, 1473, 1427, 1309, 1110, 739, 700, 607, 503. ESIMS:  $m/z$  calcd for  $[\text{C}_{29}\text{H}_{33}\text{ClNaO}_5\text{SSi}]^+$ : 579.1399, found: 579.1401.

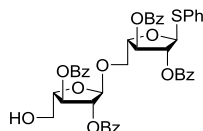
**(127) Phenyl 5-*O*-*tert*-butyldiphenylsilyl-3-*O*-chloroacetyl-1-thio- $\alpha$ -L-arabinofuranoside**



Compound **127** was furnished as a byproduct in the synthesis of compound **126** as a colorless oil.

$R_f$  0.53 (10:1 toluene-EtOAc);  $^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.70 - 7.65 (m, 4H), 7.53 - 7.49 (m, 2H), 7.47 - 7.37 (m, 6H), 7.35 - 7.26 (m, 3H), 5.63 (d,  $J = 1.4$  Hz, 1H, H-1), 5.18 (dd,  $J = 3.1, 1.5$  Hz, 1H, H-3), 4.38 - 4.32 (m, 2H, H-2, H-4), 4.13 (d,  $J = 2.2$  Hz, 2H,  $\text{CH}_2\text{Cl}$ ), 3.92 (t,  $J = 2.5$  Hz, 2H, H-5a+b), 1.07 (s, 9H, C-( $\text{CH}_3$ )<sub>3</sub>) ppm.  $^{13}\text{C NMR}$  (101 MHz,  $\text{CDCl}_3$ )  $\delta$  167.5, 135.8, 131.6, 130.3, 130.2, 129.2, 128.2, 128.0, 127.5, 94.1 (C-1), 83.5 (C-4), 82.0 (C-3), 79.9 (C-2), 63.9 (C-5), 40.8 ( $\text{CH}_2\text{Cl}$ ), 26.9 (C-( $\text{CH}_3$ )<sub>3</sub>), 19.3 ( $\text{C}(\text{CH}_3)_3$ ) ppm. **ESIMS**:  $m/z$  calcd for  $[\text{C}_{29}\text{H}_{33}\text{ClNaO}_5\text{SSi}]^+$ : 579.1399  $[\text{M}+\text{Na}]^+$ , found: 579.1394.

**(128) Phenyl 2,3-di-*O*-benzoyl- $\alpha$ -L-arabinofuranosyl-(1 $\rightarrow$ 5)-2,3-di-*O*-benzoyl-1-thio- $\alpha$ -L-arabinofuranoside**



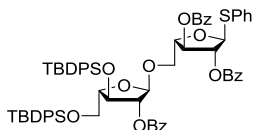
Compound **104** (50 mg, 0.111 mmol) was dissolved in anhydrous  $\text{CH}_2\text{Cl}_2$  (0.5 mL). Activated molecular sieves (100 mg) were added. The reaction mixture was cooled in a  $-78^\circ\text{C}$  bath. NIS (17 mg, 0.75 mmol) and AgOTf (14 mg, 0.55 mmol) were added. The reaction was quenched with  $\text{Et}_3\text{N}$  after 4.5 hours, afterwards it was diluted with  $\text{CH}_2\text{Cl}_2$  (2 mL) and filtered through Celite, and concentrated to give 34 mg of a clear oil. The crude product was purified on silica gel (3:1 heptane-EtOAc) to afford 8 mg of the title product as a colorless oil (18% yield). The NMR data was in accordance with reported data for compound D-**128**.<sup>207</sup>

$R_f$  0.17 (2:1 heptane-EtOAc);  $^1\text{H NMR}$  (300 MHz,  $\text{CDCl}_3$ )  $\delta$  8.12 - 7.98 (m, 7H), 7.94 - 7.89 (m, 2H), 7.63 - 7.35 (m, 15H), 7.30 - 7.27 (m, 1H), 5.82 - 5.80 (m, 1H), 5.75 - 5.70 (m, 2H), 5.64 (d,  $J = 1.4$  Hz, 1H),



5.45 - 5.42 (m, 1H), 5.40 (s, 1H), 4.74 - 4.68 (m, 1H), 4.51 - 4.45 (m, 1H), 4.24 (dd,  $J = 11.2$ , 4.1 Hz, 1H), 3.99 (dd,  $J = 11.2$ , 3.0 Hz, 3H) ppm. Compound **128** is not characterized further, since only a very small amount was prepared, and a different target disaccharide was found later.

**(130) Phenyl (2-*O*-benzoyl-3,5-di-*O*-(*tert*-butyldiphenylsilyl)- $\alpha$ -L-arabino-furanosyl)-(1 $\rightarrow$ 5)-2,3-di-*O*-benzoyl-1-thio- $\alpha$ -L-arabinofuranoside**



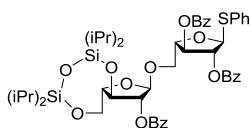
Diol **112** (100 mg, 0.289 mmol) was dissolved in anhydrous DMF (1 mL) under careful heating. Imidazole (67 mg, 1.0 mmol) was added and the reaction was cooled in a  $-78\text{ }^{\circ}\text{C}$  bath. TBDPSCl (0.150 mL, 0.577 mmol) was added and the reaction mixture was allowed to slowly heat to  $22\text{ }^{\circ}\text{C}$ . After 19 hours the reaction came to completion and was successively washed with a solution of sat. aq.  $\text{NH}_4\text{Cl}$  (25 mL), 0.5 M HCl (25 mL), a solution of sat. aq.  $\text{NaHCO}_3$  (25 mL), and water (25 mL). It was dried ( $\text{MgSO}_4$ ), filtered, and concentrated before it was purified on silica gel (20:1 heptane, EtOAc) to get Phenyl 2-*O*-benzoyl-3,5-di-*O*-(*tert*-butyldiphenylsilyl)-1-thio- $\alpha$ -L-arabinofuranoside (**129**) as 130 mg colorless oil (55% yield). Analytical data is in accordance with data for compound D-**129** ( $R_f$  0.50 (20:1 petroleumether-EtOAc),  $[\alpha]_D^{20} + 67.1$  (c 1.40,  $\text{CHCl}_3$ )).<sup>178</sup>  $R_f$  0.40 (10:1 heptane-EtOAc);  $^1\text{H NMR}$  (300 MHz,  $\text{CDCl}_3$ )  $\delta$  7.73 (d,  $J = 8.3$  Hz, 2H), 7.65 - 7.52 (m, 10H), 7.41 - 7.14 (m, 18H), 5.51 (d,  $J = 2.0$  Hz, 1H), 5.41 (t,  $J = 1.8$  Hz, 1H), 4.59 - 4.45 (m, 2H), 3.72 (dd,  $J = 11.4$ , 2.4 Hz, 1H), 3.62 (dd,  $J = 11.4$ , 4.7 Hz, 1H), 1.08 (s, 9H), 0.94 (s, 9H), C-( $\text{CH}_3$ )<sub>3</sub> ppm.

The two glycosides **104** (46.5 mg, 0.103 mmol) and **129** (100 mg, 0.121 mmol) were dissolved in anhydrous  $\text{CH}_2\text{Cl}_2$  (1 mL) under argon. 100 mg activated molecular sieves (4 Å) were added and the mixture was cooled in a  $-78\text{ }^{\circ}\text{C}$  bath. TESOTf (27  $\mu\text{L}$ , 0.121 mmol) dissolved in anhydrous  $\text{CH}_2\text{Cl}_2$  (200  $\mu\text{L}$ ) and NIS (27 mg, 0.121 mmol) were added and the reaction was stirred for 1 hour, before it was quenched with  $\text{Et}_3\text{N}$  (0.1 mL). The molecular sieves were filtered off and washed with 1 mL  $\text{CH}_2\text{Cl}_2$ . The combined organic phases were washed with 10% aq.  $\text{Na}_2\text{S}_2\text{O}_3$  (2 mL) and water (3 x 2 mL), dried ( $\text{MgSO}_4$ ), filtered, and

concentrated. The crude product was purified by flash chromatography (20:1 heptane-EtOAc) to get 60 mg colorless oil.

$R_f$  0.11 (10:1 heptane-EtOAc);  $[\alpha]_D^{20}$  - 61.4 ( $c$  1.0  $\text{CHCl}_3$ ).  $^1\text{H NMR}$  (300 MHz,  $\text{CDCl}_3$ )  $\delta$  8.12 - 8.06 (m, 4H), 7.71 (d,  $J$  = 8.3 Hz, 2H), 7.61 - 7.52 (m, 12H), 7.52 - 7.09 (m, 24H), 5.80 (d,  $J$  = 1.0 Hz, 1H, H-1), 5.74 - 5.71 (m, 2H, H-2, H-3), 5.32 (d,  $J$  = 2.1 Hz, 1H, H-2'), 5.09 (s, 1H, H-1'), 4.80 (q,  $J$  = 4.8 Hz, 1H, H-4), 4.51 (dd,  $J$  = 6.1, 2.1 Hz, 1H, H-3'), 4.38 - 4.30 (m, 1H, H-4'), 4.13 (dd,  $J$  = 11.3, 4.8 Hz, 1H, H-5a), 3.97 (dd,  $J$  = 11.3, 4.8 Hz, 1H, H-5b), 3.68 (dd,  $J$  = 11.5, 2.5 Hz, 1H, H-5'a), 3.56 (dd,  $J$  = 11.5, 4.5 Hz, 1H, H-5'b), 0.96 (s, 9H, C-( $\text{CH}_3$ )<sub>3</sub>), 0.93 (s, 9H, C-( $\text{CH}_3$ )<sub>3</sub>) ppm.  $^{13}\text{C NMR}$  (101 MHz,  $\text{CDCl}_3$ )  $\delta$  165.5, 165.5, 165.3 (2C), 136.0, 135.9, 135.8, 135.7, 133.7, 133.6, 133.5, 133.3, 133.1, 132.9, 132.1, 130.2, 129.9, 129.9, 129.6, 129.4, 129.3, 129.1, 128.7, 128.6, 128.2, 127.8, 127.7, 127.7, 106.5 (C-1'), 91.3 (C-1), 85.2 (C-2'), 84.9 (C-4'), 82.2 (C-2), 82.0 (C-4), 77.9 (C-3), 77.0 (C-3'), 66.5 (C-5), 62.8 (C-5'), 26.9 (C-( $\text{CH}_3$ )<sub>3</sub>), 26.9 (C-( $\text{CH}_3$ )<sub>3</sub>), 19.4 (C-( $\text{CH}_3$ )<sub>3</sub>), 19.2 (C-( $\text{CH}_3$ )<sub>3</sub>) ppm. **IR** (neat,  $\text{cm}^{-1}$ ): 3070, 2930, 2857, 1722, 1427, 1261, 1104, 1068, 1025, 700, 503. **ESIMS**:  $m/z$  calcd for  $[\text{C}_{69}\text{H}_{70}\text{NaO}_{11}\text{SSi}_2]^+$ : 1185.4070, found: 1185.4082.

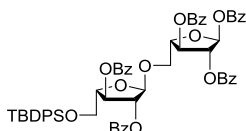
**(131) Phenyl (2-*O*-benzoyl-3,5-*O*-(1,1,3,3-tetraisopropylidisiloxane-1,3-diyl)- $\alpha$ -L-arabinofuranosyl)-(1 $\rightarrow$ 5)-2,3-di-*O*-benzoyl-1-thio- $\alpha$ -L-arabinofuranoside**



The two thioglycosides **104** (42 mg, 93  $\mu\text{mol}$ ) and **111** (50 mg, 85  $\mu\text{mol}$ ) were dissolved in anhydrous  $\text{CH}_2\text{Cl}_2$  (0.5 mL) under argon. 100 mg activated molecular sieves (4 Å) were added and the mixture was cooled in a  $-78^\circ\text{C}$  bath. TESOTf (19  $\mu\text{L}$ , 85  $\mu\text{mol}$ ) and NIS (19 mg, 85  $\mu\text{mol}$ ) were added, and the reaction was stirred for 3.5 hours before it was quenched with  $\text{Et}_3\text{N}$  (10  $\mu\text{L}$ ). The molecular sieves were filtered off and washed with 1 mL  $\text{CH}_2\text{Cl}_2$ . The combined organic phases were washed with 10% aq.  $\text{Na}_2\text{S}_2\text{O}_3$  (2 mL) and water (2 mL), dried ( $\text{MgSO}_4$ ), filtered, and concentrated. The crude product was purified by flash chromatography (heptane) to get 28 mg colorless oil.

**R<sub>f</sub>** 0.36 (6:1 heptane-EtOAc); **<sup>1</sup>H NMR** (300 MHz, CDCl<sub>3</sub>) δ 8.16 - 8.08 (m, 2H), 8.07 - 7.99 (m, 4H), 7.64 - 7.39 (m, 11H), 7.33 - 7.22 (m, 3H), 5.73 - 5.67 (m, 1H), 5.57 - 5.50 (m, 2H), 5.32 (d, *J* = 2.2 Hz, 1H), 4.62 - 4.44 (m, 2H), 4.27 - 4.17 (m, 1H), 4.11 - 3.88 (m, 5H), 1.20 - 0.80 (m, 28H, *i*-Pr). **<sup>13</sup>C NMR** (75 MHz, CDCl<sub>3</sub>) δ 165.7, 165.7, 165.6, 134.8, 133.7, 133.6, 133.4, 132.3, 131.6, 130.3, 130.2, 130.0, 129.9, 129.5, 129.2, 129.1, 128.7, 128.7, 128.6, 127.5, 101.5, 89.5, 84.7, 83.4, 81.7, 81.1, 76.5, 75.5, 61.9, 61.4, 17.7, 17.6, 17.2, 17.2, 17.2, 17.1, 13.7, 13.4, 13.2, 13.1, 13.1, 12.8, 12.7. **IR** (neat, cm<sup>-1</sup>): 2867, 1738, 1464, 1217, 1132, 1078, 1043, 886, 770.

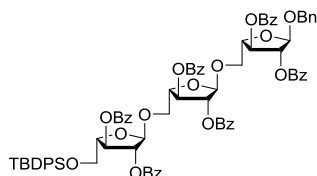
**(137) 2,3-Di-*O*-benzoyl-5-*O*-(*tert*-butyldiphenylsilyl)-β-*L*-arabinofuranosyl-(1→5)-1,2,3-tri-*O*-benzoyl-α-*L*-arabinofuranose**



By-product obtained as a clear oil in the large-scale synthesis of disaccharide donor **99**.

**R<sub>f</sub>** 0.11 (2:1 heptane-EtOAc); **<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>) δ 8.00 (d, *J* = 7.4 Hz, 5H), 7.90 (dd, *J* = 14.3, 7.4 Hz, 6H), 7.82 (d, *J* = 7.4 Hz, 2H), 7.63 - 7.56 (m, 5H), 7.52 - 7.17 (m, 30H), 6.59 (s, 1H, H-1), 5.69 (s, 1H, H-2), 5.65 (d, *J* = 3.6 Hz, 1H, H-3), 5.52 (d, *J* = 4.7 Hz, 1H, H-3'), 5.43 (s, 1H, H-2'), 5.27 (s, 1H, H-1'), 4.63 (q, *J* = 3.9 Hz, 1H, H-4), 4.39 (q, *J* = 4.5 Hz, 1H, H-4'), 4.14 (dd, *J* = 11.1, 4.7 Hz, 1H, H-5a), 3.91 - 3.80 (m, 3H, H-5b, H-5'), 0.92 (s, 9H, TBDPS) ppm. **<sup>13</sup>C NMR** (101 MHz, CDCl<sub>3</sub>) δ 165.6, 165.6, 165.4, 165.2, 164.8, 135.8, 135.8, 133.8, 133.8, 133.7, 133.5, 133.4, 133.3, 133.3, 130.3, 130.1, 130.1, 130.0, 130.0, 129.8, 129.6, 129.4, 129.3, 129.3, 128.8, 128.7, 128.7, 128.6, 128.6, 128.5, 128.4, 127.8, 106.3 (C-1'), 100.0 (C-1), 84.9 (C-4), 83.5 (C-4'), 82.2 (C-2'), 80.7 (C-2), 77.4 (C-3'), 77.3 (C-3), 66.3 (C-5), 63.5 (C-5'), 26.9 (TBPDS), 19.4 (TBDPS) ppm.

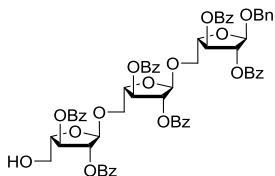
**(139) Benzyl (2,3-di-*O*-benzoyl-5-*O*-(*tert*-butyldiphenylsilyl)- $\alpha$ -L-arabinofuranosyl)-(1 $\rightarrow$ 5)-(2,3-di-*O*-benzoyl- $\alpha$ -L-arabinofuranosyl)-(1 $\rightarrow$ 5)-2,3-di-*O*-benzoyl- $\alpha$ -L-arabinofuranoside**



Disaccharide donor **99** (5.9 g, 5.83 mmol) and acceptor **89** (2.4 g, 5.45 mmol) were dissolved in anhydrous  $\text{CH}_2\text{Cl}_2$  (250 mL) and stirred with 7.5 mg activated molecular sieves (4 Å) and NIS (1.3 g, 5.83 mmol) for 1 hour at 24 °C before the mixture was cooled in a - 78 °C bath. TfOH (96.4  $\mu\text{L}$ , 1.1 mmol) was added and the reaction was stirred for 5 hours slowly raising the temperature to - 35 °C, before TLC (1:1 heptane-EtOAc) indicated that the reaction was done.  $\text{Et}_3\text{N}$  (1 mL) was added and stirred for 15 minutes before 10% aq.  $\text{Na}_2\text{S}_2\text{O}_3$  (100 mL) was added. After another 20 minutes the molecular sieves were filtered off and the organic phase was washed with water (2 x 200 mL), dried ( $\text{MgSO}_4$ ), filtrated, and concentrated. Purification by automated flash chromatography (heptane-EtOAc, 20%  $\rightarrow$  40% EtOAc) resulted in the title product as 5.4 g colorless oil (74% yield).

$R_f$  0.64 (1:1 heptane-EtOAc);  $[\alpha]_D^{20}$  - 11.7 ( $c$  1.0  $\text{CHCl}_3$ ).  $^1\text{H NMR}$  (300 MHz,  $\text{CDCl}_3$ )  $\delta$  8.07 - 7.85 (m, 12H), 7.74 - 7.64 (m, 4H), 7.60 - 7.19 (m, 29H), 5.67 - 5.60 (m, 4H, H-3, H-3', H-3'', H-2''), 5.59 (d,  $J$  = 1.3 Hz, 1H, H-2'), 5.56 (d,  $J$  = 1.3 Hz, 1H, H-2), 5.39 (s, 1H, H-1'), 5.38 (s, 1H, H-1''), 5.32 (s, 1H, H-1), 4.83 (d,  $J$  = 12.0 Hz, 1H,  $\text{CH}_2\text{Ph}$ ), 4.67 - 4.62 (m, 1H, H-4'), 4.59 (d,  $J$  = 12.0 Hz, 1H,  $\text{CH}_2\text{Ph}$ ), 4.53 - 4.44 (m, 2H, H-4'', H-4), 4.20 (dd,  $J$  = 11.6, 5.6 Hz, 2H, H-5a, H-5'a), 3.99 - 3.89 (m, 4H, H-5b, H-5'b, H-5''a+b), 1.03 (s, 9H, C-( $\text{CH}_3$ )<sub>3</sub>) ppm.  $^{13}\text{C NMR}$  (75 MHz,  $\text{CDCl}_3$ )  $\delta$  165.7, 165.7, 165.6, 165.3, 165.5, 165.3, 135.8, 133.4, 133.3, 133.1, 130.1, 130.0, 129.9, 129.9, 129.7, 129.4, 129.2, 128.6, 128.5, 128.5, 128.4, 128.3, 127.9, 127.8, 106.1 (C-1'), 105.9 (C-1''), 104.9 (C-1), 82.3 (C-4'), 82.3 (4C, C-2, C-2'', C-4, C-4''), 81.8 (C-2'), 77.5 (3C, C-3, C-3', C-3''), 68.7 ( $\text{CH}_2\text{Ph}$ ), 66.1 (C-5), 66.0 (C-5'), 63.5 (C-5''), 26.9 (C-( $\text{CH}_3$ )<sub>3</sub>), 19.4 (C-( $\text{CH}_3$ )<sub>3</sub>) ppm.  $\text{IR}$  (neat,  $\text{cm}^{-1}$ ): 3065, 2931, 2858, 1718, 1602, 1452, 1248, 1096, 1069, 1025, 704. **ESIMS**:  $m/z$  calcd for  $[\text{C}_{80}\text{H}_{74}\text{NaO}_{19}\text{Si}]^+$ : 1389.4486  $[\text{M}+\text{Na}]^+$ , found: 1389.4470.

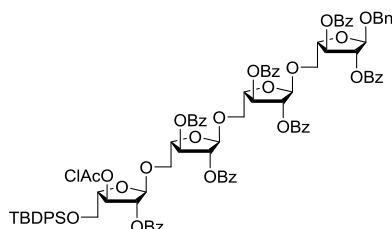
**(140) Benzyl (2,3-di-*O*-benzoyl- $\alpha$ -L-arabinofuranosyl)-(1 $\rightarrow$ 5)-(2,3-di-*O*-benzoyl- $\alpha$ -L-arabinofuranosyl)-(1 $\rightarrow$ 5)-2,3-di-*O*-benzoyl- $\alpha$ -L-arabinofuranoside**



Trisaccharide **139** (3.5 g, 2.56 mmol) was dried under vacuum and dissolved in anhydrous THF (35 mL). Acetic Acid (249 mL, 4.35 mmol) and TBAF (1M in THF) (2.94 mL, 2.94 mmol) were added. The reaction was stirred at 25 °C for 20 hours at which point TCL showed complete conversion of the starting material. The reaction mixture was diluted with CH<sub>2</sub>Cl<sub>2</sub> (5 mL) and washed with a solution of sat. aq. NH<sub>4</sub>Cl (50 mL), brine (50 mL), and water (50 mL). The organic phase was dried (MgSO<sub>4</sub>), filtered, and concentrated to give 3.8 g of a yellow oil that was dispersed on 10 g silica and subjected to automated flash chromatography (heptane-EtOAc, 40%  $\rightarrow$  45% EtOAc) to afford 2.6 g title product as a fluffy white solid (90% yield).

**R<sub>f</sub>** 0.15 (2:1 heptane-EtOAc); [ $\alpha$ ]<sub>D</sub><sup>20</sup> - 8.9 (*c* 1.0 CHCl<sub>3</sub>); **mp.** 77.9 - 81.5 °C. **<sup>1</sup>H NMR** (300 MHz, CDCl<sub>3</sub>)  $\delta$  8.11 - 7.97 (m, 8H), 7.89 (dd, *J* = 6.9, 1.2 Hz, 4H), 7.61 - 7.48 (m, 4H), 7.47 - 7.36 (m, 12H), 7.35 - 7.19 (m, 7H), 5.67 - 5.60 (m, 4H, H-2', H-2'', H-3, H-3'), 5.59 (d, *J* = 1.2 Hz, 1H, H-2), 5.44 - 5.39 (m, 3H, H-1', H-1'', H-3''), 5.32 (s, 1H, H-1), 4.83 (d, *J* = 12.0 Hz, 1H, CH<sub>2</sub>Ph), 4.66 - 4.56 (m, 2H, CH<sub>2</sub>Ph, H-4''), 4.50 - 4.43 (m, 2H, H-4, H-4'), 4.24 - 4.16 (m, 2H, H-5a, H-5'a), 4.05 - 3.88 (m, 4H, H-5b, H-5'b, H-5''a+b) ppm. **<sup>13</sup>C NMR** (101 MHz, CDCl<sub>3</sub>)  $\delta$  166.2, 165.8, 165.8, 165.5, 165.3, 165.3, 137.5, 133.7, 133.6, 133.5, 133.4, 133.4, 130.1, 130.0, 130.0, 129.9, 129.4, 129.3, 129.2, 129.2, 129.1, 128.7, 128.6, 128.5, 128.5, 128.4, 127.9, 127.9, 106.0, 105.9 (C1', C1''), 105.0 (C1), 83.8, 82.3 (C4, C4'), 82.2 (C4''), 81.9, 81.8 (C2', C2''), 81.7 (C2), 77.8 (C3''), 77.4 (2C, C3, C3'), 68.7 (CH<sub>2</sub>Ph), 66.3, 66.2 (C5, C5'), 62.5 (C5'') ppm. **IR** (neat, cm<sup>-1</sup>): 2930, 1716, 1602, 1452, 1248, 1106, 1069, 10246, 961, 706. **ESIMS**: *m/z* calcd for [C<sub>64</sub>H<sub>56</sub>NaO<sub>19</sub>]<sup>+</sup>: 1151.3308 [M+Na]<sup>+</sup>, found: 1151.3329.

**(141) Benzyl (2-*O*-benzoyl-5-*O*-(*tert*-butyldiphenylsilyl)-3-*O*-chloroacetyl- $\alpha$ -L-arabinofuranosyl)-(1 $\rightarrow$ 5)-(2,3-di-*O*-benzoyl- $\alpha$ -L-arabinofuranosyl)-(1 $\rightarrow$ 5)-(2,3-di-*O*-benzoyl- $\alpha$ -L-arabinofuranosyl)-(1 $\rightarrow$ 5)-2,3-di-*O*-benzoyl- $\alpha$ -L-arabinofuranoside**

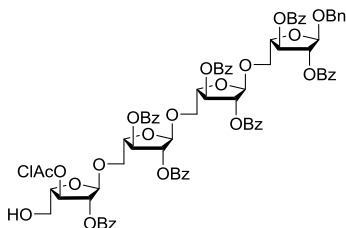


A solution of acceptor **140** (2.2 g, 1.98 mmol) and donor **87** (1.6 g, 2.42 mmol) were concentrated from toluene and dried under vacuum for 16 hours. The starting materials were dissolved in anhydrous  $\text{CH}_2\text{Cl}_2$  (64 mL) under  $\text{N}_2$  and stirred with 7.6 g molecular sieves (4 Å) at 22 °C for 30 minutes before cooled in a – 65 °C bath. TfOH (43  $\mu\text{L}$ , 0.48 mmol) and NIS (544 mg, 2.4 mmol) were added. The reaction came to completion within 30 minutes, so it was quenched with  $\text{Et}_3\text{N}$  (2.2 mL) and heated to 22 °C. 10% aq.  $\text{Na}_2\text{S}_2\text{O}_3$  (50 mL) was added, and the mixture was stirred for 1 hour. The molecular sieves were filtered off, and the two phases were separated. The organic solution was washed with water (2 x 50 mL), dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and concentrated to give 4.7 g of a yellow oil that was dispersed on 15 g silica and subjected to automated flash chromatography (heptane-EtOAc, 20%  $\rightarrow$  30% EtOAc) on a 40 g column. The title product was obtained as 2.81 g fluffy white solid (86% yield). *Note: Avoid quenching with  $\text{Et}_3\text{N}$ , as it removes the chloroacetic acid group. Instead, use the workup procedure for compound 147.*

$R_f$  0.53 (1:1 heptane-EtOAc);  $[\alpha]_D^{20}$  - 18.9 ( $c$  1.0  $\text{CHCl}_3$ ); **mp.** 75.4 - 76.6 °C.  **$^1\text{H}$  NMR** (400 MHz,  $\text{CDCl}_3$ )  $\delta$  8.15 - 8.07 (m, 2H), 8.05 - 7.96 (m, 6H), 7.95 - 7.84 (m, 6H), 7.71 - 7.60 (m, 4H), 7.60 - 7.15 (m, 32H), 5.69 - 5.55 (m, 6H, H-2, H-2', H-2'', H-3, H-3', H-3''), 5.44 (s, 1H, H-1'''), 5.41 - 5.36 (m, 3H, H-1, H-1', H-2''', H-3'''), 5.33 (s, 1H, H-1'), 5.32 (s, 1H, H-1''), 4.83 (d,  $J$  = 11.9 Hz, 1H,  $\text{CH}_2\text{Ph}$ ), 4.66 - 4.52 (m, 3H,  $\text{CH}_2\text{Ph}$ , H-4', H-4''), 4.50 - 4.43 (m, 1H, H-4), 4.27 - 4.14 (m, 3H, H-4''', H-5'a, H-5''a), 4.08 (dd,  $J$  = 11.4, 3.8 Hz, 1H, H-5a), 3.98 - 3.80 (m, 6H, 1  $\times$   $\text{CH}_2\text{Cl}$ , H-5b, H-5'b, H-5''b, H-5'''a+b), 3.71 (d,  $J$  = 15.4 Hz, 1H, 1  $\times$   $\text{CH}_2\text{Cl}$ ), 0.97 (s, 9H, C-( $\text{CH}_3$ )<sub>3</sub>) ppm.  **$^{13}\text{C}$  NMR** (101 MHz,  $\text{CDCl}_3$ )  $\delta$  166.8, 165.8, 165.8, 165.7, 165.5, 165.4, 165.3 (2C), 138.0, 137.5, 135.8, 135.7, 133.8, 133.6, 133.5, 133.4, 133.3, 133.2, 130.1, 130.1, 130.0, 130.0, 129.9, 129.8, 129.5, 129.2, 128.9,

128.7, 128.6, 128.6, 128.6, 128.5, 128.0, 127.9, 127.9, 127.9, 125.4, 106.0 (C-1'''), 105.9 (C-1), 105.5 (C-1''), 105.0 (C-1'), 83.4 (C-4'''), 82.3 (C-4), 82.2 (C-4''), 82.1 (C-2'''), 82.0 (C-4'), 81.9, 81.8, 81.7 (C-2-C-2''), 78.5 (C-3'''), 77.4 (C-3'), 77.3 (C-3), 76.9 (C-3''), 68.7 ( $\underline{\text{CH}_2\text{Ph}}$ ), 66.1, 66.0 (C-5, C-5'), 65.2 (C-5''), 63.2 (C-5'''), 40.8 ( $\text{CH}_2\text{Cl}$ ), 26.9 (C-( $\underline{\text{CH}_3}$ )<sub>3</sub>), 19.5 ( $\underline{\text{C}}\text{-(CH}_3\text{)}_3$ ) ppm. **IR** (neat,  $\text{cm}^{-1}$ ): 2927, 2855, 23389, 1719, 1602, 1452, 1250, 1105, 1069, 1025, 963, 705. **ESIMS**:  $m/z$  calcd for  $[\text{C}_{94}\text{H}_{87}\text{ClNaO}_{25}\text{Si}]^+$ : 1701.4886  $[\text{M}+\text{Na}]^+$ , found: 1701.4877.

**(142) Benzyl (2-*O*-benzoyl-3-*O*-chloroacetyl- $\alpha$ -L-arabinofuranosyl)-(1 $\rightarrow$ 5)-(2,3-di-*O*-benzoyl- $\alpha$ -L-arabinofuranosyl)-(1 $\rightarrow$ 5)-(2,3-di-*O*-benzoyl- $\alpha$ -L-arabinofuranosyl)-(1 $\rightarrow$ 5)-2,3-di-*O*-benzoyl- $\alpha$ -L-arabinofuranoside**

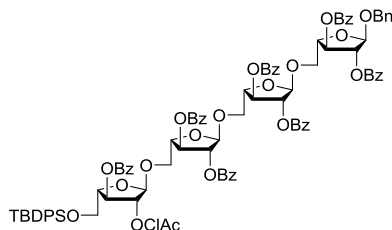


To a solution of tetrasaccharide **141** (2.72 g, 1.62 mmol) in  $\text{CH}_2\text{Cl}_2$  (27 mL) and MeCN (80 mL) in a plastic flask was added HF (1 mL, 70% in pyridine) and one drop of water. The reaction was stirred at 22 °C for 24 hours before TLC showed complete conversion of the starting material.  $\text{Me}_3\text{SiOMe}$  (12 mL, 84 mmol) was added and the reaction mixture was stirred for 20 minutes before it was diluted with EtOAc (50 mL), washed with a solution of sat. aq.  $\text{NaHCO}_3$  (3 x 50 mL) and dried ( $\text{Na}_2\text{SO}_4$ ). The organic solution was concentrated to give 10 g of a fluffy white solid of which some was dissolvable in  $\text{CH}_2\text{Cl}_2$ . White crystals were filtered off, and the solution was concentrated to afford 3.6 g of a fluffy white solid that was dispersed in 15 g silica and purified on a 40 g column by automated flash chromatography (heptane-EtOAc, 40%  $\rightarrow$  45% EtOAc). The title compound was obtained as a fluffy white solid in 78% yield (1.83 g).

**R<sub>f</sub>** 0.31 (1:1 heptane-EtOAc);  $[\alpha]_{\text{D}}^{20}$  -23.5 ( $c$  1.0  $\text{CHCl}_3$ ); **mp.** 78.6 - 78.9 °C. **<sup>1</sup>H NMR** (400 MHz,  $\text{CDCl}_3$ )  $\delta$  8.14 - 8.08 (m, 2H), 8.04 - 7.96 (m, 8H), 7.94 - 7.87 (m, 4H), 7.66 - 7.17 (m, 26H), 5.68 - 5.62 (m, 4H, H-2', H-3, H-3', H-3''), 5.60 (d,  $J$  = 1.2 Hz, 1H, H-2''), 5.59 (d,  $J$  = 1.0 Hz, 1H, H-2'''), 5.44 (s,

1H, H-1''), 5.41 (d,  $J = 0.7$  Hz, 1H, H-2), 5.40 (s, 1H, H-1'), 5.34 (s, 1H, H-1'''), 5.32 (s, 1H, H-1), 5.16 (d,  $J = 4.5$  Hz, 1H, H-3'''), 4.83 (d,  $J = 11.9$  sHz, 1H,  $\text{CH}_2\text{Ph}$ ), 4.66 - 4.54 (m, 3H, H-4, H-4'',  $\text{CH}_2\text{Ph}$ ), 4.50 - 4.45 (m, 1H, H-4'), 4.28 - 4.23 (m, 1H, H-4'''), 4.20 (dd,  $J = 11.2$ , 4.5 Hz, 2H, H-5'a, H-5''a), 4.04 (dd,  $J = 11.1$ , 3.8, 1H, H-5a), 3.99 - 3.81 (m, 6H,  $1 \times \text{CH}_2\text{Cl}$ , H-5b, H-5'b, H-5''b, H-5'''a+b), 3.76 (d,  $J = 15.5$  Hz, 1H,  $\text{CH}_2\text{Cl}$ ) ppm.  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$  167.5, 165.9, 165.9 (2C), 165.6, 165.4, 165.3 (2C), 137.6, 133.9, 133.9, 133.7, 133.6, 133.5, 133.5, 130.1, 130.1, 130.0, 130.0, 129.5, 129.4, 129.4, 129.1, 128.9, 128.8, 128.7, 128.7, 128.6, 128.6, 128.5, 127.9, 106.1 (C-1'), 106.0 (C-1''), 105.5 (C-1'''), 105.0 (C-1), 84.0 (C-4'''), 82.4 (C-4'), 82.6 (C-4''), 82.3 (C-2''), 82.2 (C-2'''), 81.9 (C-2'), 81.8 (C-4), 81.4 (C-2), 79.1 (C-3'''), 77.5, 77.4, 77.4 (C-3, C-3', C-3''), 68.8 ( $\text{CH}_2\text{Ph}$ ), 66.2, 66.2 (C-5', C-5''), 65.3 (C-5), 62.3 (C-5'''), 40.7 ( $\text{CH}_2\text{Cl}$ ) ppm. IR (neat,  $\text{cm}^{-1}$ ): 1717, 1452, 1249, 1106, 707. ESIMS:  $m/z$  calcd for  $[\text{C}_{78}\text{H}_{69}\text{ClNaO}_{25}]^+$ : 1463.3709  $[\text{M}+\text{Na}]^+$ , found: 1463.3710.

**(143) Benzyl (3-*O*-benzoyl-5-*O*-(*tert*-butyldiphenylsilyl)-2-*O*-chloroacetyl- $\alpha$ -L-arabinofuranosyl)-(1 $\rightarrow$ 5)-(2,3-di-*O*-benzoyl- $\alpha$ -L-arabinofuranosyl)-(1 $\rightarrow$ 5)-(2,3-di-*O*-benzoyl- $\alpha$ -L-arabinofuranosyl)-(1 $\rightarrow$ 5)-2,3-di-*O*-benzoyl- $\alpha$ -L-arabinofuranoside**



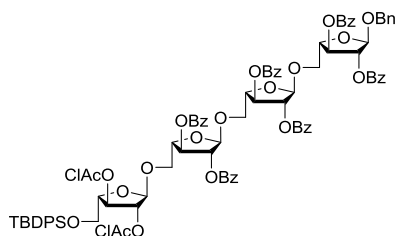
A mixture of trisaccharide acceptor **140** (867 mg, 0.768 mmol) and monosaccharide **100** (540 mg, 0.817 mmol) were dried azeotropically with toluene and subjected to high vacuum for 16 hours. The starting materials were dissolved in anhydrous  $\text{CH}_2\text{Cl}_2$  (22 mL) under  $\text{N}_2$  and stirred with 1.5 g molecular sieves (4 Å) at 22 °C for 30 minutes. NIS (183 mg, 0.817 mmol) was added and the reaction mixture was cooled in a - 65 °C bath before TfOH (14.5  $\mu\text{L}$ , 0.163 mmol) was added. The reaction came to an end within 30 minutes, then it was quenched with  $\text{Et}_3\text{N}$  (450 mL) and heated to 22 °C. 10% aq.  $\text{Na}_2\text{S}_2\text{O}_3$  (20 mL) was added and the mixture was stirred for 1 hour. The molecular sieves were filtered off and the two phases were separated. The



organic solution was washed with water (2 x 20 mL), dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and concentrated to give 1.3 g of a yellow oil that was dispersed on 6 g silica and subjected to automated flash chromatography (heptane-EtOAc, 30%  $\rightarrow$  40% EtOAc) on a 25 g column. The title product was obtained as 1.04 g fluffy white solid (81% yield). *Note: Avoid quenching with  $\text{Et}_3\text{N}$ , as it removes the chloroacetic acid group. Instead use the workup procedure for compound 147.*

$R_f$  0.48 (1:1 heptane-EtOAc);  $[\alpha]_D^{20}$  -27.6 ( $c$  1.0  $\text{CHCl}_3$ ); **mp.** 76.4 - 78.8 °C.  **$^1\text{H}$  NMR** (400 MHz,  $\text{CDCl}_3$ )  $\delta$  8.04 - 7.98 (m, 5H), 7.95 - 7.83 (m, 7H), 7.70 - 7.64 (m, 4H), 7.58 - 7.20 (m, 34H), 5.65 - 5.62 (m, 3H, H-2', H-3, H-3'), 5.61 (d,  $J$  = 1.0 Hz, 1H, H-2'), 5.60 - 5.57 (m, 2H, H-2', H-3''), 5.49 (d,  $J$  = 5.3 Hz, 1H, H-3'''), 5.39 (s, 2H, H-2''', H-1'/H-1''), 5.38 (s, 1H, H-1'/H-1''), 5.32 (s, 1H, H-1), 5.26 (s, 1H, H-1'''), 4.83 (d,  $J$  = 12.0 Hz, 1H,  $\text{CH}_2\text{Ph}$ ), 4.62 - 4.54 (m, 3H,  $\text{CH}_2\text{Ph}$ , H-4, H-4''), 4.49 - 4.45 (m, 1H, H-4'), 4.44 - 4.40 (m, 1H, H-4'''), 4.22 - 4.15 (m, 2H, H-5'a, H-5'a), 4.15 - 4.09 (m, 1H, H-5'a), 3.98, 3.96 (AB spin,  $J_{AB}$  = 15.1 Hz, 2H,  $\text{CH}_2\text{Cl}$ ), 3.95 - 3.84 (m, 5H, H-5b, H-5'b, H-5''b, H-5'''a, H-5'''b), 1.03 (s, 9H, C-( $\text{CH}_3$ )<sub>3</sub>) ppm.  **$^{13}\text{C}$  NMR** (101 MHz,  $\text{CDCl}_3$ )  $\delta$  166.2, 165.8, 165.8, 165.7, 165.7, 165.5, 165.3, 165.2, 137.5, 135.8, 135.8, 133.5, 133.5, 133.4, 133.3, 133.3, 130.1, 130.0, 129.9, 129.8, 129.4, 129.2, 129.2, 128.6, 128.6, 128.5, 128.4, 128.4, 127.9, 127.8, 106.0, 105.9 (C1', C1''), 105.5 (C1'''), 105.0 (C1), 83.2 (C2'''), 83.0 (C4'''), 82.3, 82.2 (C4, C4'), 82.2 (C4''), 81.9, 81.7, 81.6 (C2, C2', C2''), 77.5, 77.4 (C3, C3'), 77.2 (C3''), 68.7 ( $\text{CH}_2\text{Ph}$ ), 66.1, 66.0, 65.8 (C5, C5', C5''), 63.1 (C5'''), 40.7 ( $\text{CH}_2\text{Cl}$ ), 26.9 (C-( $\text{CH}_3$ )<sub>3</sub>), 19.5 (C-( $\text{CH}_3$ )<sub>3</sub>) ppm. **IR** (neat,  $\text{cm}^{-1}$ ): 2931, 1718, 1452, 1249, 1105, 1069, 1025, 962, 705. **ESIMS**:  $m/z$  calcd for  $[\text{C}_{94}\text{H}_{87}\text{ClNaO}_{25}\text{Si}]^+$ : 1701.4886  $[\text{M}+\text{Na}]^+$ , found: 1701.4891.

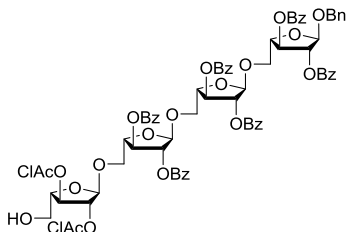
**(144) Benzyl (5-*O*-(*tert*-butyldiphenylsilyl)-2,3-di-*O*-chloroacetyl)- $\alpha$ -L-arabinofuranosyl)-(1 $\rightarrow$ 5)-(2,3-di-*O*-benzoyl- $\alpha$ -L-arabinofuranosyl)-(1 $\rightarrow$ 5)-(2,3-di-*O*-benzoyl- $\alpha$ -L-arabinofuranosyl)-(1 $\rightarrow$ 5)-2,3-di-*O*-benzoyl- $\alpha$ -L-arabinofuranoside**



Trisaccharide acceptor **140** (320 mg, 0.284 mmol) and monosaccharide **101** (200 mg, 0.316 mmol) were coevaporated with toluene and dried under vacuum for 16 hours. The starting materials were dissolved in anhydrous  $\text{CH}_2\text{Cl}_2$  (12 mL) under  $\text{N}_2$  and stirred with 0.75 g molecular sieves (4 Å) at 22 °C for 30 minutes. NIS (71 mg, 0.316 mmol) was added and the reaction mixture was cooled in a – 78 °C bath before TfOH (5.6  $\mu\text{L}$ , 63  $\mu\text{mol}$ ) was added. The reaction came to completion within 50 minutes, then it was quenched with  $\text{Et}_3\text{N}$  (180 mL), and heated to 22 °C. 10% aq.  $\text{Na}_2\text{S}_2\text{O}_3$  (10 mL) was added and the mixture was stirred for 30 minutes. The molecular sieves were filtered off and the two phases were separated. The organic solution was washed with water (2 x 50 mL), dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and concentrated to give 520 mg of a yellow oil that was dispersed on 4 g silica and subjected to automated flash chromatography (heptane-EtOAc, 25%  $\rightarrow$  35% EtOAc) on a 12 g column. The title product was obtained as 430 mg fluffy white solid (91% yield). *Note: Avoid quenching with  $\text{Et}_3\text{N}$ , as it removes the chloroacetic acid group. Instead use the workup procedure for compound 147.*

$R_f$  0.15 (2:1 heptane-EtOAc);  $[\alpha]_D^{20}$  - 30.1 (c 0.94  $\text{CHCl}_3$ ); **mp.** 74.1 - 76.8 °C.  **$^1\text{H}$  NMR** (400 MHz,  $\text{CDCl}_3$ )  $\delta$  8.08 (d,  $J$  = 7.3 Hz, 2H), 8.04 - 7.97 (m, 6H), 7.92 - 7.84 (m, 4H), 7.68 - 7.63 (m, 4H), 7.63 - 7.43 (m, 7H), 7.43 - 7.26 (m, 19H), 7.25 - 7.20 (m, 3H), 5.67 - 5.62 (m, 3H, H-2', H-3', H-3''), 5.59 (s, 2H, H2'), 5.57 (d,  $J$  = 4.1 Hz, 1H, H-3''), 5.43 (s, 1H, H-1'/H-1''), 5.40 (s, 1H, H-1'/H-1''), 5.32 (s, 1H, H-1), 5.29 (d,  $J$  = 4.9 Hz, 1H, H-3'''), 5.22 (s, 2H, H-1''', H-2'''), 4.83 (d,  $J$  = 12.0 Hz, 1H, 1  $\times$   $\text{CH}_2\text{Ph}$ ), 4.64 - 4.57 (m, 2H, H4, 1  $\times$   $\text{CH}_2\text{Ph}$ ), 4.53 (q,  $J$  = 4.1 Hz, 1H, H-4''), 4.50 - 4.45 (m, 1H, H-4'), 4.22 - 4.16 (m, 3H, H-4''', H-5a, H-5'a), 4.04 (dd,  $J$  = 11.2, 4.1 Hz, 1H, H-5''a), 3.94 (dt,  $J$  = 11.0, 2.8 Hz, 2H, H-5b, H-5'b), 3.90 - 3.87 (m, 4H, H-5'''a+b,  $\text{CH}_2\text{Cl}$ ), 3.87 - 3.78 (m, 2H, H-5''b, 1  $\times$   $\text{CH}_2\text{Cl}$ ), 3.69 (d,  $J$  = 15.4 Hz, 1H, 1  $\times$   $\text{CH}_2\text{Cl}$ ), 1.03 (s, 9H, C-( $\text{CH}_3$ )<sub>3</sub>) ppm.  **$^{13}\text{C}$  NMR** (101 MHz,  $\text{CDCl}_3$ )  $\delta$  166.9, 166.3, 165.8, 165.8, 165.7, 165.5, 165.3, 165.3, 137.6, 135.8, 133.6, 133.4, 133.2, 130.1, 129.9, 129.2, 128.8, 128.7, 128.6, 128.5, 128.4, 127.9, 127.8, 106.0, 105.9 (C-1', C-1''), 105.0 (C-1), 104.9 (C-1'''), 83.3 (C-4'''), 83.0 (C-2'''), 82.3, 82.2 (C-4, C-4'), 82.0 (C-4''), 81.9 (2C, C-2', C-2''), 81.7 (C-2), 78.1 (C-3'''), 77.4 (2C, C-3, C-3'), 68.7 ( $\underline{\text{CH}_2\text{Ph}}$ ), 66.2 (2C, C-5, C-5'), 65.1 (C-5''), 62.8 (C-5'''), 40.6 ( $\underline{\text{CH}_2\text{Cl}}$ ), 40.5 ( $\underline{\text{CH}_2\text{Cl}}$ ), 26.9 (C-( $\underline{\text{CH}_3}$ )<sub>3</sub>), 19.5 ( $\underline{\text{C}}$ -( $\text{CH}_3$ )<sub>3</sub>) ppm. **IR** (neat,  $\text{cm}^{-1}$ ): 2932, 1718, 1602, 1452, 1249, 1105, 1069, 1025, 963, 705. **ESIMS**:  $m/z$  calcd for  $[\text{C}_{89}\text{H}_{84}\text{Cl}_2\text{NaO}_{25}\text{Si}]^+$ : 1673.4340  $[\text{M}+\text{Na}]^+$ , found: 1673.4340.

**(145) Benzyl (2,3-di-*O*-chloroacetyl- $\alpha$ -L-arabinofuranosyl)-(1 $\rightarrow$ 5)-(2,3-di-*O*-benzoyl- $\alpha$ -L-arabinofuranosyl)-(1 $\rightarrow$ 5)-(2,3-di-*O*-benzoyl- $\alpha$ -L-arabinofuranosyl)-(1 $\rightarrow$ 5)-2,3-di-*O*-benzoyl- $\alpha$ -L-arabinofuranoside**

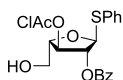


Tetrasaccharide **144** (404 mg, 0.244 mmol) was dissolved in  $\text{CH}_2\text{Cl}_2$  (4 mL) and MeCN (12 mL) in a plastic flask. 0.300 mL HF (70% in pyridine) and one drop of water were added and the reaction was stirred at 22 °C for 7 days, before TLC showed complete conversion of the starting material.  $\text{Me}_3\text{SiOMe}$  (1.8 mL, 12.6 mmol) was added and the reaction mixture was stirred for 20 minutes before it was diluted with EtOAc (3 mL), washed with a solution of sat. aq.  $\text{NaHCO}_3$  (2 x 25 mL) and water (25 mL) and dried ( $\text{Na}_2\text{SO}_4$ ). The organic solution was concentrated to give 800 mg of a white oil that was dispersed in 2.5 g silica and subjected to automated flash chromatography (heptane-EtOAc, 40%  $\rightarrow$  50% EtOAc) on a 12 g column. The title compound was obtained as a white oil in 64% yield (223 mg).

$R_f$  0.25 (1:1 heptane-EtOAc);  $[\alpha]_D^{20}$  - 25.5 ( $c$  .91  $\text{CHCl}_3$ ); **mp.** 68.6 - 69.6 °C.  **$^1\text{H}$  NMR** (400 MHz,  $\text{CDCl}_3$ )  $\delta$  8.12 - 8.06 (m, 2H), 8.04 - 7.98 (m, 6H), 7.93 - 7.87 (m, 4H), 7.63 - 7.53 (m, 2H), 7.53 - 7.44 (m, 5H), 7.44 - 7.35 (m, 8H), 7.35 - 7.23 (m, 8H), 5.67 - 5.62 (m, 3H, H-2, H-3, H-3'), 5.61 - 5.58 (m, 3H, H-2', H-2'', H-3''), 5.43 (s, 1H, H-1'), 5.40 (s, 1H, H-1''), 5.33 (s, 1H, H-1), 5.24 (d,  $J$  = 0.9 Hz, 1H, H-2'''), 5.23 (s, 1H, H-1'''), 5.05 (dd,  $J$  = 4.8, 0.9 Hz, 1H, H-3'''), 4.83 (d,  $J$  = 11.9 Hz, 1H,  $\text{CH}_2\text{Ph}$ ), 4.64 - 4.57 (m, 2H,  $\text{CH}_2\text{Ph}$ , H-4), 4.53 (dt,  $J$  = 5.1, 3.2 Hz, 1H, H-4''), 4.47 (dt,  $J$  = 4.7, 3.0 Hz, 1H, H-4'), 4.23 - 4.20 (m, 2H, H-4''', H-5a/H-5'a), 4.18 (dd,  $J$  = 4.3, 1.8 Hz, 1H, H-5a/H-5'a), 4.07 - 4.02 (m, 3H,  $\text{CH}_2\text{Cl}$ , H-5''a), 3.98 - 3.92 (m, 2H, H-5b, H-5'b), 3.92 - 3.77 (m, 4H, 1  $\times$   $\text{CH}_2\text{Cl}$ , H-5'''a+b, H-5''b), 3.73 (d,  $J$  = 15.4 Hz, 1H, 1  $\times$   $\text{CH}_2\text{Cl}$ ) ppm.  **$^{13}\text{C}$  NMR** (101 MHz,  $\text{CDCl}_3$ )  $\delta$  167.3, 166.1, 165.9 (2C), 165.9, 165.5, 165.3 (2C), 137.5, 133.9, 133.6, 133.5, 133.4, 130.1, 130.0, 129.9, 129.4, 129.3, 129.2, 129.1, 128.9, 128.7, 128.6, 128.6, 128.5, 128.5, 127.9, 105.9 (2C, C-1', C-1''), 105.0 (C-1), 104.9 (C-1'''), 83.5 (C-4'''), 82.4 (C-2'''), 82.3, 82.2 (C-4, C-4'), 82.1, 81.9 (C-2', C-2''), 81.8 (C-2), 81.7 (C-4''), 78.4 (C-3'''), 77.4 (2C, C-3, C-3'), 76.8 (C-3''), 68.8 ( $\text{CH}_2\text{Ph}$ ),

66.2, 66.1 (C-5, C-5'), 65.3 (C-5''), 61.8 (C-5'''), 40.6 ( $\underline{\text{CH}_2\text{Cl}}$ ), 40.5 ( $\underline{\text{CH}_2\text{Cl}}$ ) ppm. **IR** (neat,  $\text{cm}^{-1}$ ): 2927, 1717, 1452, 1248, 1106, 707. **ESIMS**:  $m/z$  calcd for  $[\text{C}_{73}\text{H}_{66}\text{Cl}_2\text{NaO}_{25}]^+$ : 1435.3162  $[\text{M}+\text{Na}]^+$ , found: 1435.3164.

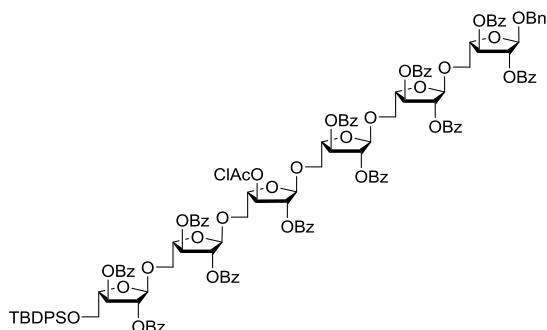
**(146) Phenyl 2-*O*-benzoyl-3-*O*-chloroacetyl-1-thio- $\alpha$ -L-arabinofuranoside**



A solution of compound **87** (100 mg, 151  $\mu\text{mol}$ ) in  $\text{CH}_2\text{Cl}_2$  (7.5 mL) and MeCN (22 mL) in a plastic flask was cooled to 0 °C. HF (755  $\mu\text{L}$ , 50% in water) was added, and the reaction was stirred at 22 °C for 24 hours before TLC showed complete conversion of the starting material.  $\text{Me}_3\text{SiOMe}$  (7.5 mL, 54 mmol) was added and the reaction mixture was stirred for 1 hour. Afterwards the reaction mixture was successively washed with a solution of sat. aq.  $\text{NaHCO}_3$  (2x 35 mL) and dried ( $\text{Na}_2\text{SO}_4$ ). The aqueous solution was extracted with  $\text{CH}_2\text{Cl}_2$  (2 x 75 mL), and the combined organic phases were dried ( $\text{Na}_2\text{SO}_4$ ) and concentrated to give 600 mg of a milky liquid. The solution was dispersed on 1 g Celite and purified on a dry column. The title compound was obtained as a clear oil in 72% yield (50 mg).

$R_f$  0.44 (10:1 heptane-EtOAc),  **$^1\text{H}$  NMR** (400 MHz,  $\text{CDCl}_3$ )  $\delta$  8.02 (dd,  $J = 5.2$ , 3.3 Hz, 2H), 7.64 - 7.56 (m, 1H), 7.56 - 7.51 (m, 2H), 7.45 (t,  $J = 7.7$  Hz, 2H), 7.37 - 7.27 (m, 3H), 5.74 - 5.71 (m, 1H, H-1), 5.56 (t,  $J = 1.8$  Hz, 1H, H-3), 5.40 (dd,  $J = 5.3$ , 1.4 Hz, 1H, H-2), 4.50 - 4.45 (m, 1H, H-4), 4.19 (s, 2H,  $\underline{\text{CH}_2\text{Cl}}$ ), 4.04 - 3.89 (m, 2H, H-5a+b) ppm.  **$^{13}\text{C}$  NMR** (101 MHz,  $\text{CDCl}_3$ )  $\delta$  167.0, 165.4, 133.9, 132.4, 130.0, 129.3, 128.7, 128.1, 91.2 (C-1), 82.9 (C-4), 82.2 (C-2), 78.8 (C-3), 61.6 (C-5), 40.7 ( $\underline{\text{CH}_2\text{Cl}}$ ) ppm. **IR** (neat,  $\text{cm}^{-1}$ ): 1718, 1019, 753, 683.

**(147) Benzyl (2,3-di-*O*-benzoyl-5-*O*-(*tert*-butyldiphenylsilyl)- $\alpha$ -L-arabinofuranosyl)-(1 $\rightarrow$ 5)-(2,3-di-*O*-benzoyl- $\alpha$ -L-arabinofuranosyl)-(1 $\rightarrow$ 5)-(2-*O*-benzoyl-3-*O*-chloroacetyl- $\alpha$ -L-arabinofuranosyl)-(1 $\rightarrow$ 5)-(2,3-di-*O*-benzoyl- $\alpha$ -L-arabinofuranosyl)-(1 $\rightarrow$ 5)-(2,3-di-*O*-benzoyl- $\alpha$ -L-arabinofuranosyl)-(1 $\rightarrow$ 5)-2,3-di-*O*-benzoyl- $\alpha$ -L-arabinofuranoside**

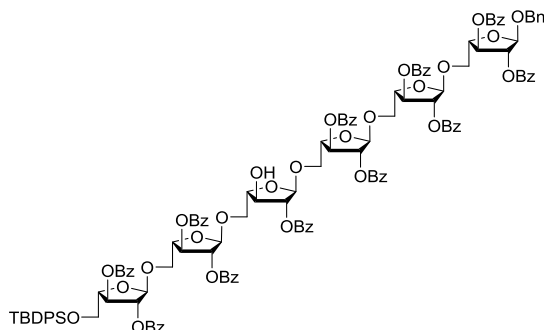


Tetrasaccharide acceptor **142** (150 mg, 0.104 mmol) and disaccharide donor **99** (160 mg, 0.155 mmol) were mixed, concentrated from toluene, and dried under vacuum for 16 hours. The starting materials and NIS (40 mg, 0.180 mmol) were dissolved in anhydrous  $\text{CH}_2\text{Cl}_2$  (7.5 mL) under  $\text{N}_2$  and stirred with 1 g molecular sieves (4 Å) at 22 °C for 30 minutes before cooled in a - 70 °C bath. TfOH (3.2  $\mu\text{L}$ , 36  $\mu\text{mol}$ ) was added and the reaction came to completion within 50 minutes under which the temperature was raised to - 60 °C. The molecular sieves were filtered off and the reaction mixture was poured into a mixture of 10% aq.  $\text{Na}_2\text{S}_2\text{O}_3$  (1 mL) and sat. aq.  $\text{NaHCO}_3$  (1 mL). The organic layer was washed with water (2 x 2 mL), dried ( $\text{Na}_2\text{SO}_4$ ), filtered and concentrated to give 280 mg of a yellow oil that was dispersed on 2.5 g silica and subjected to automated flash chromatography (heptane-EtOAc, 30%  $\rightarrow$  40% EtOAc) on a 12 g column. The title product was obtained as 200 mg fluffy white solid (80% yield).

$R_f$  0.54 (1:1 heptane-EtOAc);  $[\alpha]_D^{20}$  - 22.5 ( $c$  1.0  $\text{CHCl}_3$ ); mp. 91 - 93 °C.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  8.14 - 8.10 (m, 2H), 8.04 - 7.79 (m, 20H), 7.71 - 7.65 (m, 4H), 7.65 - 7.19 (m, 44H), 5.68 - 5.62 (m, 4H, H-3, H-3', H-3'', H-2'), 5.62 - 5.57 (m, 4H, H-3''', H-3''', H-2'', H-2), 5.55 (s, 1H, H-2'''), 5.54 (s, 1H, H-2'''), 5.45 (s, 1H, H-2''), 5.44 (s, 1H, H-1'), 5.40 (s, 1H, H-1'), 5.37 (d,  $J$  = 5.0 Hz, 1H, H-3'''), 5.36 (s, 1H, H-1'), 5.34 - 5.30 (m, 3H, H-1, H-1', H-1'), 4.83 (d,  $J$  = 12.0 Hz, 1H,  $\text{CH}_2\text{Ph}$ ), 4.65 - 4.62 (m, 2H, H-4',  $\text{CH}_2\text{Ph}$ ), 4.57 - 4.43 (m, 4H, 4 x H-4), 4.35 - 4.31 (m, 1H, H-4''), 4.20 (dd,  $J$  = 11.2, 4.5, 2H, 2 x H-5a), 4.18 - 4.03 (m, 3H, 3 x H-5a), 3.98 - 3.76 (m, 8H,

1  $\times$  CH<sub>2</sub>Cl, H-5''''''a, 6  $\times$  H-5b), 3.68 (d,  $J$  = 15.3 Hz, 1H, 1  $\times$  CH<sub>2</sub>Cl), 1.00 (s, 9H, C-(CH<sub>3</sub>)<sub>3</sub>) ppm. <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$  167.0, 165.8, 165.8, 165.7, 165.6, 165.6, 165.5, 165.4, 165.3 (2C), 165.2 (2C), 137.6, 135.8, 135.8, 133.6, 133.5, 133.4, 130.1, 130.0, 130.0, 129.9, 129.8, 129.4, 129.2, 128.8, 128.6, 128.6, 128.5, 128.5, 128.4, 128.4, 128.3, 127.9, 127.8, 127.8, 106.1 (C-1<sup>?</sup>), 105.9 (3  $\times$  C-1), 105.3 (C-1<sup>?</sup>), 105.0 (C-1), 83.3 (C-4'''''''), 82.3 (3C), 82.2, 82.2 (2C), 82.1, 81.9, 81.8 (2C) (5  $\times$  C-4, 5  $\times$  C-2), 81.1 (C-2'''''''), 78.5 (C-3'''''), 77.4 (5C, 5  $\times$  C-3), 68.7 (CH<sub>2</sub>Ph), 66.1 (C-5<sup>?</sup>), 66.0 (C-5<sup>?</sup>), 65.9 (C-5<sup>?</sup>), 65.5 (C-5<sup>?</sup>), 64.9 (C-5<sup>?</sup>), 63.5 (C-5'''''''), 40.5 (CH<sub>2</sub>Cl), 32.0, 26.9 (C-(CH<sub>3</sub>)<sub>3</sub>), 19.4 (C-(CH<sub>3</sub>)<sub>3</sub>) ppm. IR (neat, cm<sup>-1</sup>): 2930, 1718, 1601, 1451, 1248, 1096, 1069, 1025, 962, 705. ESIMS:  $m/z$  calcd for [C<sub>132</sub>H<sub>119</sub>ClNaO<sub>37</sub>Si]<sup>+</sup>: 2381.6780 [M+Na]<sup>+</sup>, found: 2381.6577.

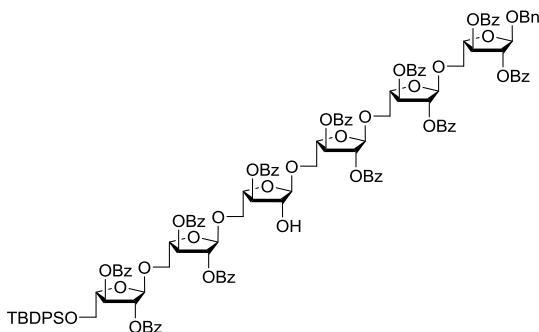
**(148) Benzyl (2,3-di-*O*-benzoyl-5-*O*-(*tert*-butyldiphenylsilyl)- $\alpha$ -L-arabinofuranosyl)-(1 $\rightarrow$ 5)-(2,3-di-*O*-benzoyl- $\alpha$ -L-arabinofuranosyl)-(1 $\rightarrow$ 5)-(2-*O*-benzoyl- $\alpha$ -L-arabinofuranosyl)-(1 $\rightarrow$ 5)-(2,3-di-*O*-benzoyl- $\alpha$ -L-arabinofuranosyl)-(1 $\rightarrow$ 5)-(2,3-di-*O*-benzoyl- $\alpha$ -L-arabinofuranosyl)-(1 $\rightarrow$ 5)-2,3-di-*O*-benzoyl- $\alpha$ -L-arabinofuranoside**



Hexasaccharide **147** (300 mg, 0.127 mmol) was dissolved in 1:1 mixture of MeOH and THF (4 mL). Et<sub>3</sub>N (17.7  $\mu$ L, 0.127 mmol) was added to the reaction mixture and stirred for 24 hours at 20  $^{\circ}$ C. The reaction mixture was diluted with CH<sub>2</sub>Cl<sub>2</sub> (1 mL) and washed with brine (5 mL) and water (5 mL). The organic phase was dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated to give 290 mg of a fluffy white solid that was concentrated on silica and subjected to automated flash chromatography (heptane-EtOAc, 35%  $\rightarrow$  40% EtOAc) on a 12 g silica column. The title product was received as 222 mg fluffy white solid (77% yield).

**R<sub>f</sub>** 0.50 (1:1 heptane-EtOAc);  $[\alpha]_{\text{D}}^{20}$  -24.6 °C (*c* 1.0 CHCl<sub>3</sub>); **mp.** 92.1 - 95.1 °C. **<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>) δ 8.08 - 7.85 (m, 21H), 7.72 - 7.65 (m, 4H), 7.58 - 7.46 (m, 6H), 7.46 - 7.20 (m, 39H), 5.65 - 5.58 (m, 7H, H-3'''''), 3 × H-3, 3 × H-2), 5.54 (s, 2H, 2 × H-2), 5.52 (d, *J* = 5.1 Hz, 1H, H-3<sup>3</sup>), 5.42 (s, 1H, H-1<sup>3</sup>), 5.40 (s, 1H, H-1<sup>3</sup>), 5.36 (s, 1H, H-1'''), 5.34 (s, 1H, H-1<sup>3</sup>), 5.32 (s, 1H, H-1), 5.32 (s, 1H, H-1<sup>3</sup>), 5.16 (d, *J* = 2.3 Hz, 1H, H-2'''), 4.83 (d, *J* = 12.0 Hz, 1H, CH<sub>2</sub>Ph), 4.64 - 4.57 (m, 2H, CH<sub>2</sub>Ph, H-4<sup>3</sup>), 4.54 (q, *J* = 4.0 Hz, 1H, H-4<sup>3</sup>), 4.51 - 4.45 (m, 3H, H-4'''''), 2 × H-4), 4.39, 4.37 (AB spin, *J*<sub>AB</sub> = 4.3 Hz, 1H, H-4'''), 4.25 (dd, *J* = 5.6, 2.3 Hz, 1H, H-3'''), 4.23 - 4.08 (m, 4H, 4 × H-5a), 4.03 - 3.91 (m, 5H, H-5''''')<sup>a+b</sup>, H-5''')<sup>a</sup>, 2 × H-5b), 3.88 (dd, *J* = 11.1, 2.7 Hz, 2H, 2 × H-5b), 3.80 (dd, *J* = 11.3, 3.8 Hz, 1H, H-5''')<sup>b</sup>, 1.00 (s, 9H, C-(CH<sub>3</sub>)<sub>3</sub>) ppm. **<sup>13</sup>C NMR** (101 MHz, CDCl<sub>3</sub>) δ 166.7 (3C), 165.8, 165.7, 165.6, 165.5, 165.4, 165.4, 165.3, 165.3, 137.5, 135.8, 135.8, 133.6, 133.3, 130.1, 130.1, 130.0, 130.0, 129.9, 129.8, 129.4, 129.3, 129.2, 129.2, 128.6, 128.6, 128.5, 128.5, 128.4, 128.4, 128.4, 127.9, 127.8, 106.1 (C-1<sup>3</sup>), 106.1 (C-1<sup>3</sup>), 106.0 (C-1<sup>3</sup>), 105.9 (C-1<sup>3</sup>), 105.4 (C-1'''), 105.0 (C-1), 85.8 (C-2'''), 83.3 (C-4'''''), 83.0 (C-4'''), 82.3 (4C, C-2<sup>3</sup>, 3 × C-4), 81.9 (C-4<sup>3</sup>), 81.9 (C-2<sup>3</sup>), 81.9 (C-2<sup>3</sup>), 81.7 (C-2<sup>3</sup>), 81.6 (C-2<sup>3</sup>), 77.4 (5C, 5 × C-2), 76.7 (C-3'''), 68.7 (CH<sub>2</sub>Ph), 66.2 (2C, 2 × C-5), 66.0 (3C, C-5''', 2 × C-5), 63.5 (C-5'''''), 26.9 (C-(CH<sub>3</sub>)<sub>3</sub>), 19.4 (C-(CH<sub>3</sub>)<sub>3</sub>) ppm. **IR** (neat, cm<sup>-1</sup>): 2930, 1720, 1602, 1263, 1107, 1026, 963, 707. **ESIMS**: *m/z* calcd for [C<sub>130</sub>H<sub>118</sub>NaO<sub>36</sub>Si]<sup>+</sup>: 2305.7064 [M+Na]<sup>+</sup>, found: 2305.6898.

**(150) Benzyl (2,3-di-*O*-benzoyl-5-*O*-(*tert*-butyldiphenylsilyl)- $\alpha$ -L-arabinofuranosyl)-(1 $\rightarrow$ 5)-(2,3-di-*O*-benzoyl- $\alpha$ -L-arabinofuranosyl)-(1 $\rightarrow$ 5)-(3-*O*-benzoyl- $\alpha$ -L-arabinofuranosyl)-(1 $\rightarrow$ 5)-(2,3-di-*O*-benzoyl- $\alpha$ -L-arabinofuranosyl)-(1 $\rightarrow$ 5)-2,3-di-*O*-benzoyl- $\alpha$ -L-arabinofuranoside**



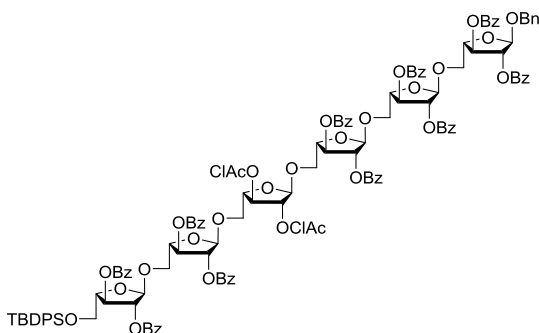
A solution of tetrasaccharide **88** (1.44 g, 0.696 mmol) and disaccharide **99** (930 mg, 0.904 mmol) were coevaporated with toluene and dried under vacuum for 16 hours. The starting materials were dissolved in anhydrous  $\text{CH}_2\text{Cl}_2$  (37 mL) under  $\text{N}_2$  and stirred with 3 g molecular sieves (4 Å) and NIS (203 mg, 0.904 mmol) at 22 °C for 30 minutes. The reaction mixture was cooled in a – 55 °C bath and TfOH (16  $\mu\text{L}$ , 0.181 mmol) was added. The reaction came to completion within 2.5 hours, under which the temperature was raised to – 35 °C. The molecular sieves were filtered off and the reaction mixture was poured into a mixture of 10% aq.  $\text{Na}_2\text{S}_2\text{O}_3$  (20 mL) and a solution of sat. aq.  $\text{NaHCO}_3$  (20 mL), shaken, and separated. The organic solution was washed with water (2 x 40 mL), dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and concentrated to give 1.8 g of a yellow oil that was dissolved in 1:1 mixture of MeOH and THF (23 mL).  $\text{Et}_3\text{N}$  (125  $\mu\text{L}$ , 0.900 mmol) was added to the reaction mixture that was stirred for 24 hours at 20 °C. The reaction mixture was diluted with EtOAc (20 mL) and washed with brine (2 x 60 mL) and water (60 mL). The organic phase was dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and concentrated to afford 1.9 g of a fluffy white solid that was concentrated on silica and subjected to automated flash chromatography (heptane-EtOAc, 35%  $\rightarrow$  40% EtOAc) on a 26 g silica column. The title product was received as 1.22 g fluffy white solid (71% yield over two steps).

$R_f$  0.47 (1:1 heptane-EtOAc);  $[\alpha]_D^{20}$  – 25.9 ( $c$  1.0  $\text{CHCl}_3$ ); **mp.** 95.9 – 97.4 °C.  **$^1\text{H}$  NMR** (400 MHz,  $\text{CDCl}_3$ )  $\delta$  8.10 (d,  $J$  = 7.2 Hz, 2H), 8.04 – 7.83 (m, 19H), 7.74 – 7.67 (m, 4H), 7.59 – 7.43 (m, 8H), 7.43 – 7.20 (m, 36H), 5.71 (d,  $J$  = 5.0 Hz, 1H, H-3''/H-3'''), 5.66 – 5.60 (m, 4H, H-2''', H-3''', 2  $\times$  H-3), 5.60 – 5.54 (m, 4H, H-3', 3  $\times$  H-2), 5.51 (d,  $J$  = 1.2 Hz, 1H, H-2''/H-2'''), 5.39 (s, 1H, H-1'), 5.38 (s, 1H, H-1'), 5.36 (s, 1H, H-1'), 5.35 (s, 1H, H-1'), 5.32 (s, 1H, H-1), 5.26 (s, 1H, H-1''), 5.22 (d,  $J$  = 4.1 Hz, 1H, H-3''), 4.83 (d,  $J$  = 12.0 Hz, 1H,  $\text{CH}_2\text{Ph}$ ), 4.63 – 4.57 (m, 2H,  $\text{CH}_2\text{Ph}$ , H-4'), 4.56 – 4.49 (m, 3H, H-4''', 2H-4), 4.49 – 4.45 (m, 2H, H-2''), 4.28 (d,  $J$  = 12.0 Hz, 1H, H-2'''), 4.23 – 4.06 (m, 5H, 5  $\times$  H-5a), 4.02 – 3.95 (m, 2H, H-5'''), 3.95 – 3.83 (m, 5H, 5  $\times$  H-5b), 3.44 (d,  $J$  = 9.0 Hz, 1H, OH), 1.01 (s, 9H,  $\text{C}(\text{CH}_3)_3$ ).  **$^{13}\text{C}$  NMR** (101 MHz,  $\text{CDCl}_3$ )  $\delta$  166.6, 165.9, 165.8, 165.8, 165.7, 165.6, 165.6, 165.5, 165.3, 165.3, 165.2, 137.5, 135.8, 135.8, 133.5, 133.3, 133.2, 130.3, 130.1, 130.1, 130.0, 129.9, 129.8, 129.8, 129.4, 129.2, 128.6, 128.6, 128.5, 128.5, 128.4, 128.3, 127.9, 127.8, 127.8, 108.5 (C-1''), 106.3 (C-1'), 106.1 (C-1'), 105.9 (C-1'), 105.9 (C-1'), 105.0 (C-1), 83.4 (C-4'), 82.5 (4C, C-4), 82.3 (2C, C-4', C-2'), 82.1 (C-2''/C-2'''), 81.9 (2C, C-2), 81.7 (C-2'), 80.4 (C-3''), 79.8 (C-2'''), 77.4 (3C, 3  $\times$  C-3), 77.3 (C-3'), 77.0 (C-3''/C-3'''), 68.7 ( $\text{CH}_2\text{Ph}$ ), 66.2 (C-5') 66.1 (2C, C-5), 66.1 (C-5'), 65.9 (C-5'), 65.8 (C-5'),



63.6 (C-5'''''), 26.9 (C-(CH<sub>3</sub>)<sub>3</sub>), 19.4 (C-(CH<sub>3</sub>)<sub>3</sub>) ppm. **IR** (neat, cm<sup>-1</sup>): 2931, 1718, 1451, 1248, 1096, 705. **ESIMS**: *m/z* calcd for [C<sub>130</sub>H<sub>118</sub>NaO<sub>36</sub>Si]<sup>+</sup>: 2305.7064 [M+Na]<sup>+</sup>, found: 2305.6699.

**(151) Benzyl (2,3-di-*O*-benzoyl-5-*O*-(*tert*-butyldiphenylsilyl)- $\alpha$ -L-arabinofuranosyl)-(1 $\rightarrow$ 5)-(2,3-di-*O*-benzoyl- $\alpha$ -L-arabinofuranosyl)-(1 $\rightarrow$ 5)-(2,3-di-*O*-chloroacetyl- $\alpha$ -L-arabinofuranosyl)-(1 $\rightarrow$ 5)-(2,3-di-*O*-benzoyl- $\alpha$ -L-arabinofuranosyl)-(1 $\rightarrow$ 5)-(2,3-di-*O*-benzoyl- $\alpha$ -L-arabinofuranosyl)-(1 $\rightarrow$ 5)-2,3-di-*O*-benzoyl- $\alpha$ -L-arabinofuranoside**

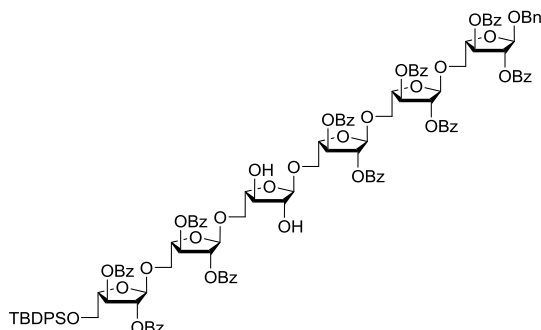


A mixture of tetrasaccharide **145** (210 mg, 0.148 mmol) and disaccharide **99** (200 mg, 0.193 mmol) were coevaporated with toluene and dried under vacuum for 16 hours. The starting materials were dissolved in anhydrous CH<sub>2</sub>Cl<sub>2</sub> (7 mL) under N<sub>2</sub> and stirred with 800 mg molecular sieves (4 Å) and NIS (43 mg, 0.193 mmol) at 22 °C for 30 minutes. The reaction mixture was cooled in a - 78 °C bath and TfOH (4.3 µL, 48 µmol) was added. The reaction came to completion within 90 minutes, under which the temperature was raised to - 60 °C. The molecular sieves were filtered off and the reaction mixture was poured into a mixture of 10% aq. Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> (3 mL) and a solution of sat. aq. NaHCO<sub>3</sub> (3 mL), shaken and separated. The organic solution was washed with water (2 x 50 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), filtered and concentrated to give 460 mg of a yellow oil that was dispersed on 4 g silica and subjected to automated flash chromatography (heptane-EtOAc, 30%  $\rightarrow$  40% EtOAc) on a 12 g column. The title product was obtained as 257 mg fluffy white solid (74% yield).

**R<sub>f</sub>** 0.54 (1:1 heptane-EtOAc); [ $\alpha$ ]<sub>D</sub><sup>20</sup> - 21.8 (*c* .98 CHCl<sub>3</sub>); **mp.** 94.3 - 97.2 °C. **<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>)  $\delta$  8.10 - 8.06 (m, 2H), 8.04 - 7.85 (m, 16H), 7.72 - 7.66 (m, 4H), 7.63 - 7.20 (m, 44H), 5.66 - 5.57 (m, 8H, 5  $\times$  H-3, 3  $\times$  H-2), 5.56 (d, *J* = 1.3 Hz, 1H, H-2<sup>3</sup>), 5.54 (d, *J* = 1.1 Hz, 1H, H-2<sup>3</sup>), 5.42 (s,

1H, H-1<sup>3</sup>), 5.40 (s, 1H, H-1<sup>3</sup>), 5.36 (s, 1H, H-1<sup>3</sup>), 5.32 (s, 1H, H-1), 5.29 (s, 1H, H-1<sup>3</sup>), 5.24 (d,  $J = 5.0$  Hz, 1H, H-3'''), 5.20 (d,  $J = 1.2$  Hz, 1H, H-2'''), 5.19 (s, 1H, H-1'''), 4.83 (d,  $J = 12.0$  Hz, 1H, CH<sub>2</sub>Ph), 4.63 - 4.57 (m, 2H, CH<sub>2</sub>Ph, H-4<sup>3</sup>), 4.53 - 4.45 (m, 4H, 4 × H-4), 4.27 (dt,  $J = 5.0, 2.6$  Hz, 1H, H-4'''), 4.22 - 4.14 (m, 3H, 3 × H-5a), 4.05 - 3.93 (m, 7H, 2 × H-5a, H-5''''''a+b, CH<sub>2</sub>Cl, H-5<sup>3</sup>b), 3.93 - 3.89 (m, 2H, 2 × H-5b), 3.89 - 3.80 (m, 3H, 1 × CH<sub>2</sub>Cl, 2 × H-5b), 3.77 (dd,  $J = 11.1, 2.8$  Hz, 1H, H-5<sup>3</sup>b), 3.68 (d,  $J = 15.3$  Hz, 1H, 1 × CH<sub>2</sub>Cl), 1.01 (s, 9H, C-(CH<sub>3</sub>)<sub>3</sub>) ppm. <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ 166.5, 165.8, 165.8 (3C), 165.6, 165.5, 165.6, 165.3 (2C), 165.3, 137.5, 135.8, 133.6, 133.4, 133.2, 130.1, 130.1, 130.0, 129.9, 129.9, 129.8, 129.4, 129.2, 129.1, 128.8, 128.6, 128.6, 128.5, 128.5, 128.5, 128.4, 127.9, 127.8, 106.3 (C-1<sup>3</sup>), 106.1 (C-1<sup>3</sup>), 105.9 (2C, C-1<sup>3</sup>), 105.0 (C-1), 104.9 (C-1'''), 83.3 (C-4<sup>3</sup>), 82.3 (C-2'''), 82.2 (C-4<sup>3</sup>), 82.2 (C-2<sup>3</sup>), 82.2 (C-4<sup>3</sup>), 82.1 (C-4'''), 82.1 (2C, 2 × C-4), 81.9 (2C, 2 × C-2), 81.8 (C-2<sup>3</sup>), 81.4 (H-2<sup>3</sup>), 78.1 (C-3'''), 77.4 (4C, 4 × C-3), 76.8 (C-3<sup>3</sup>), 68.7 (CH<sub>2</sub>Ph), 66.0 (4C, 4C-5), 65.1 (C-5'''), 63.5 (C-5'''''), 40.6 (CH<sub>2</sub>Cl), 40.5 (CH<sub>2</sub>Cl), 26.9 (C-(CH<sub>3</sub>)<sub>3</sub>), 19.4 (C-(CH<sub>3</sub>)<sub>3</sub>) ppm. **ESIMS**:  $m/z$  calcd for [C<sub>127</sub>H<sub>116</sub>Cl<sub>2</sub>NaO<sub>37</sub>Si]<sup>+</sup>: 2353.6234 [M+Na]<sup>+</sup>, found: 2353.5833.

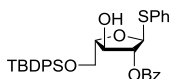
**(152) Benzyl (2,3-di-*O*-benzoyl-5-*O*-(*tert*-butyldiphenylsilyl)- $\alpha$ -L-arabinofuranosyl)-(1 $\rightarrow$ 5)-(2,3-di-*O*-benzoyl- $\alpha$ -L-arabinofuranosyl)-(1 $\rightarrow$ 5)-( $\alpha$ -L-arabinofuranosyl)-(1 $\rightarrow$ 5)-(2,3-di-*O*-benzoyl- $\alpha$ -L-arabinofuranosyl)-(1 $\rightarrow$ 5)-(2,3-di-*O*-benzoyl- $\alpha$ -L-arabinofuranosyl)-(1 $\rightarrow$ 5)-2,3-di-*O*-benzoyl- $\alpha$ -L-arabinofuranoside**



Hexasaccharide **151** (180 mg, 77  $\mu$ mol) was dissolved in 1:1 mixture of MeOH and THF (2.4 mL). Et<sub>3</sub>N (23.7  $\mu$ L, 0.169 mmol) was added to the reaction mixture that was stirred for 24 hours at 20 °C. The reaction mixture was diluted

with  $\text{CH}_2\text{Cl}_2$  (2 mL) and washed with brine (5 mL) and water (5 mL). The organic phase was dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and concentrated to afford 168 mg of a white crystals that was concentrated on silica and subjected to automated flash chromatography (heptane-EtOAc, 35%  $\rightarrow$  45% EtOAc) on a 4 g silica column. The title product was received as 118 mg fluffy white solid (70% yield).

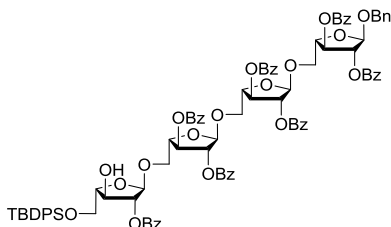
$R_f$  0.26 (1:1 heptane-EtOAc);  $[\alpha]_D^{20}$  -18.6 ( $c$  1.0  $\text{CHCl}_3$ ), **mp.** 92.3 - 95.6  $^\circ\text{C}$ .  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  8.13 (d,  $J$  = 7.4 Hz, 2H), 8.07 - 7.86 (m, 17H), 7.73 - 7.67 (m, 4H), 7.60 - 7.20 (m, 42H), 5.67 - 5.60 (m, 6H, H-2''/H-2''', H-2''', H-3''',  $3 \times$  H-3), 5.59 (d,  $J$  = 0.8 Hz, 1H, H-2'), 5.55 (d,  $J$  = 1.0 Hz, 1H, H-2'''), 5.47 (d,  $J$  = 0.7 Hz, 1H, H-2'), 5.43 - 5.39 (m, 3H, H-3''/H-3''',  $2 \times$  H-1), 5.36 (s, 1H, H-1'''), 5.32 (s, 1H, H-1), 5.28 (s, 1H, H-1'), 5.16 (s, 1H, H-1''), 4.83 (d,  $J$  = 12.0 Hz, 1H,  $\text{CH}_2\text{Ph}$ ), 4.63 - 4.57 (m, 2H,  $\text{CH}_2\text{Ph}$ , H-4), 4.52 (q,  $J$  = 3.7 Hz, 2H, H-4''', H-4''/H-4''), 4.49 - 4.42 (m, 2H,  $2 \times$  H-4'), 4.27 (d,  $J$  = 2.0 Hz, 1H, H-4''), 4.23 - 4.15 (m, 3H,  $3 \times$  H-5a), 4.08 - 4.04 (br, 2H, H-2'', H-3''), 4.04 - 3.89 (m, 7H, H-5''a, H-5''a/H-5''', H-5''', H-5''a+b,  $3 \times$  H-5b), 3.86 (dd,  $J$  = 11.0, 3.7 Hz, 1H, H-5''b/H-5'''), 3.74 (dd,  $J$  = 10.9, 2.0 Hz, 1H, H-5''b), 1.02 (s, 9H, C-( $\text{CH}_3$ )<sub>3</sub>) ppm.  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$  165.9 (2C), 165.8, 165.8, 165.6, 165.5, 165.5, 165.4, 165.3, 165.3, 137.5, 135.8, 133.7, 133.7, 133.6, 133.6, 133.5, 133.5, 133.4, 133.4, 133.3, 133.3, 130.3, 130.1, 130.1, 130.0, 130.0, 129.9, 129.8, 129.4, 129.4, 129.2, 129.2, 129.1, 129.0, 128.7, 128.7, 128.6, 128.5, 128.5, 128.4, 128.4, 127.9, 127.8, 127.8, 108.1 (C-1'''), 106.2 (C-1'), 106.1 (C-1'''), 106.0 (C-1'), 105.9 (C-1'), 105.0 (C-1), 86.0 (C-4''), 83.4 (C-4'''), 82.9 (C-4'), 82.3 (C-4'), 82.3 (C-2'''), 82.2 (C-4'), 81.9 (C-2'), 81.9 (2C, C-2''', C-2''/C-2'''), 81.8 (2C,  $2 \times$  C-2), 81.7 (C-4''/C-4'''), 79.5 (C-2''), 78.2 (C-3''), 77.4 (4C,  $4 \times$  C-3), 77.0 (C-3'), 68.7 ( $\text{CH}_2\text{Ph}$ ), 66.9 (C-5'') 66.2 (3C,  $3 \times$  C-5), 65.5 (C-5''/C-5'''), 63.6 (C-5'''), 26.9 (C-( $\text{CH}_3$ )<sub>3</sub>), 19.4 (C-( $\text{CH}_3$ )<sub>3</sub>) ppm. **IR** (neat,  $\text{cm}^{-1}$ ): 2930, 1719, 1452, 1249, 1096, 1025, 963, 706. **ESIMS**:  $m/z$  calcd for  $[\text{C}_{123}\text{H}_{114}\text{O}_{35}\text{Si}]$ : 2201.6802  $[\text{M}+\text{Na}]^+$ , found: 2201.6466.

**(153) Phenyl 2-*O*-benzoyl-5-*O*-*tert*-butyldiphenylsilyl-1-thio- $\alpha$ -L-arabino-furanoside**

Diol **112** (2.58 g, 7.43 mmol) was dissolved in anhydrous DMF (25 mL) under careful heating. Imidazole (1.79 g, 26.3 mmol) was added and the reaction was cooled in a  $-78\text{ }^{\circ}\text{C}$  bath. TBDPSCl (2.22 mL, 8.54 mmol) was added and the reaction mixture was allowed to heat to  $4\text{ }^{\circ}\text{C}$  over 16 hours. The reaction mixture was successively washed with a solution of sat. aq.  $\text{NH}_4\text{Cl}$  (25 mL), 0.5 M HCl (25 mL), a solution of sat. aq.  $\text{NaHCO}_3$  (25 mL), and water (25 mL), dried ( $\text{MgSO}_4$ ), filtered and concentrated before it was subjected to automated flash chromatography (heptane-EtOAc, 0%  $\rightarrow$  20% EtOAc) to get 2.91 g colorless oil as the title compound (67% yield).

$R_f$  0.13 (10:1 heptane-EtOAc);  $[\alpha]_D^{20}$  -106.5 ( $c$  0.98  $\text{CHCl}_3$ ).  $^1\text{H NMR}$  (300 MHz,  $\text{CDCl}_3$ )  $\delta$  8.08 - 7.99 (m, 2H), 7.75 - 7.54 (m, 7H), 7.51 - 7.28 (m, 11H), 5.77 (d,  $J = 3.8\text{ Hz}$ , 1H, H-1), 5.16 (t,  $J = 3.8\text{ Hz}$ , 1H, H-2), 4.41 - 4.34 (m, 2H, H-3, H-4), 3.98 (dd,  $J = 11.3, 3.1\text{ Hz}$ , 1H, H-5a), 3.91 (dd,  $J = 11.3, 4.0\text{ Hz}$ , 1H, H-5b), 3.35 - 3.28 (m, 1H, OH), 1.03 (s, 9H, C-( $\text{CH}_3$ )<sub>3</sub>) ppm.  $^{13}\text{C NMR}$  (75 MHz,  $\text{CDCl}_3$ )  $\delta$  167.6, 135.9, 135.82, 134.1, 133.97, 133.4, 132.2, 130.2, 130.0, 129.2, 129.1, 128.8, 127.9, 127.9, 127.8, 89.3 (C-1), 87.4 (C-2), 83.5 (C-3), 77.2 (C-4), 63.4 (C-5), 27.0 (C-( $\text{CH}_3$ )<sub>3</sub>), 19.5 (C-( $\text{CH}_3$ )<sub>3</sub>), ppm. **IR** (neat,  $\text{cm}^{-1}$ ): 3491, 3070, 2930, 2857, 1708, 1427, 1267, 1109, 1026, 740, 701, 607, 503. **ESIMS**:  $m/z$  calcd for  $[\text{C}_{34}\text{H}_{36}\text{NaO}_5\text{SSi}]^+$ : 607.1945  $[\text{M}+\text{Na}]^+$ , found: 607.1957.

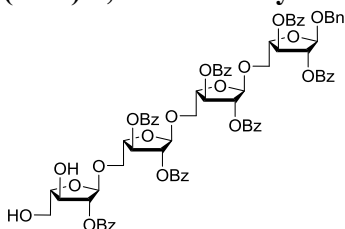
**(154) Benzyl (2-*O*-benzoyl-5-*O*-(*tert*-butyldiphenylsilyl)- $\alpha$ -L-arabinofuranosyl)-(1 $\rightarrow$ 5)-(2,3-di-*O*-benzoyl- $\alpha$ -L-arabinofuranosyl)-(1 $\rightarrow$ 5)-(2,3-di-*O*-benzoyl- $\alpha$ -L-arabinofuranosyl)-(1 $\rightarrow$ 5)-2,3-di-*O*-benzoyl- $\alpha$ -L-arabinofuranoside**



The title compound (**159**) was a fluffy white solid, obtained as a byproduct in the synthesis of compound **141**.

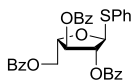
$R_f$  0.45 (1:1 heptane-EtOAc);  $[\alpha]_D^{20}$  -25.4 (c 0.95  $\text{CHCl}_3$ ); **mp.** 77.7 - 78.4 °C.  **$^1\text{H}$  NMR** (400 MHz,  $\text{CDCl}_3$ )  $\delta$  8.09 - 8.04 (m, 2H), 8.04 - 7.97 (m, 6H), 7.94 - 7.86 (m, 6H), 7.68 - 7.61 (m, 4H), 7.59 - 7.46 (m, 5H), 7.45 - 7.35 (m, 13H), 7.35 - 7.28 (m, 7H), 7.28 - 7.20 (m, 7H), 5.65 - 5.64 (m, 2H, H-2/H-2', H-3/H-3'), 5.63 (s, 1H, H-3/H-3'), 5.61 (d,  $J$  = 1.2 Hz, 1H, H-2''), 5.59 (d,  $J$  = 1.2 Hz, 1H, H-2/H-2'), 5.51 (dd,  $J$  = 5.0, 1.2 Hz, 1H, H-3''), 5.43 (s, 1H, H-1'/H-1''), 5.40 (s, 1H, H-1'/H-1''), 5.34 (s, 1H, H-1'''), 5.32 (s, 1H, H-1), 5.16 (d,  $J$  = 1.7 Hz, 1H, H-2'''), 4.83 (d,  $J$  = 11.9 Hz, 1H,  $\text{CH}_2\text{Ph}$ ), 4.64 - 4.54 (m, 3H, H-4,  $\text{CH}_2\text{Ph}$ , H-4''), 4.48 (td,  $J$  = 4.7, 3.0 Hz, 1H, H-4'), 4.28 (q,  $J$  = 4.7 Hz, 1H, H-4'''), 4.25 - 4.17 (m, 3H, H-3''', H-5a, H-5'a), 4.11 (dd,  $J$  = 11.1, 4.9 Hz, 1H, H-5''a), 3.97 - 3.88 (m, 3H, H-5b, H-5'b, H-5''b), 3.85 (d,  $J$  = 4.7 Hz, 2H, H-5'''a+b), 0.99 (s, 9H, C-( $\text{CH}_3$ )<sub>3</sub>) ppm.  **$^{13}\text{C}$  NMR** (101 MHz,  $\text{CDCl}_3$ )  $\delta$  166.5, 166.1, 165.8, 165.8, 165.5, 165.4, 165.3, 137.6, 135.8, 135.7, 133.6, 133.6, 133.5, 133.5, 133.3, 130.1, 130.1, 130.0, 129.9, 129.8, 129.4, 129.3, 129.2, 129.2, 129.2, 128.6, 128.6, 128.6, 128.5, 128.4, 127.9, 127.8, 127.8, 127.8, 106.1 (C-1), 105.9 (C-1'''), 105.4, 105.0 (C-1', C-1''), 85.5 (C-2'''), 85.2 (C-4'''), 82.3, 82.3 (C-4, C-4'), 81.9, 81.9 (C-2'', C-4''), 81.8, 81.7 (C-2, C-2'), 77.4 (3C, C-3, C-3', C-3''), 76.9 (C-3'''), 68.7 ( $\underline{\text{CH}_2\text{Ph}}$ ), 66.2, 66.2 (C-5, C-5'), 65.9 (C-5''), 63.5 (C-5'''), 26.9 (C-( $\underline{\text{CH}_3}$ )<sub>3</sub>), 19.4 ( $\underline{\text{C}}$ -( $\text{CH}_3$ )<sub>3</sub>) ppm. **IR** (neat,  $\text{cm}^{-1}$ ): 293, 171, 1452, 1249, 1096, 1069, 1025, 704. **ESIMS**:  $m/z$  calcd for  $[\text{C}_{92}\text{H}_{86}\text{NaO}_{24}\text{Si}]^+$ : 1625.5170  $[\text{M}+\text{Na}]^+$ , found: 1625.5165.

**(156) Benzyl (2-*O*-benzoyl- $\alpha$ -L-arabinofuranosyl)-(1 $\rightarrow$ 5)-(2,3-di-*O*-benzoyl- $\alpha$ -L-arabinofuranosyl)-(1 $\rightarrow$ 5)-(2,3-di-*O*-benzoyl- $\alpha$ -L-arabinofuranosyl)-(1 $\rightarrow$ 5)-2,3-di-*O*-benzoyl- $\alpha$ -L-arabinofuranoside**



Compound **154** (1.0 g, 0.624 mmol) was dissolved in anhydrous THF (24 mL) and cooled to 0 °C in an ice bath. Acetic acid (71  $\mu$ L, 1.25 mmol) and TBAF (1 M in THF) (0.748 mL, 0.748 mmol) were added to the reaction mixture. The reaction was heated to 22 °C and stirred for 24 h. The mixture was diluted with CH<sub>2</sub>Cl<sub>2</sub> (5 mL) and washed with a solution of sat. aq. NH<sub>4</sub>Cl (20 mL) and water (20 mL). The organic phase was dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated to give 970 mg of a fluffy white solid that was dispersed on 6 g silica and purified on a 12 g column by automated flash chromatography (heptane-EtOAc, 40%  $\rightarrow$  45% EtOAc). The title compound was obtained as a fluffy white solid (770 mg, 90%).

$R_f$  0.18 (1:1 heptane-EtOAc);  $[\alpha]_D^{20}$  -21.3 ( $c$  0.9 CHCl<sub>3</sub>); **mp.** 77.9 - 79.8 °C. **<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>)  $\delta$  8.10 - 7.96 (m, 10H), 7.91 (d,  $J$  = 7.6 Hz, 4H), 7.62 - 7.47 (m, 5H), 7.47 - 7.35 (m, 13H), 7.35 - 7.21 (m, 8H), 5.65 (s, 1H, H2/H2'/H2''), 5.65 (s, 1H, H-3/H-3'), 5.64 (s, 1H, H-3/H-3'), 5.61 (s, 1H, H-2'), 5.60 (s, 1H, H-2'), 5.55 (d,  $J$  = 4.8 Hz, 1H, H-3''), 5.44 (s, 1H, H-1'/H-1''), 5.41 (s, 1H, H-1'/H-1''), 5.37 (s, 1H, H-1'''), 5.33 (s, 1H, H-1), 5.13 (d,  $J$  = 2.3 Hz, 1H, H-2'''), 4.84 (d,  $J$  = 11.9 Hz, 1H, CH<sub>2</sub>Ph), 4.65 - 4.53 (m, 3H, H-4, H-4'', CH<sub>2</sub>Ph), 4.50 - 4.46 (m, 1H, H-4'), 4.31 - 4.27 (m, 1H, H-4'''), 4.21 (dd,  $J$  = 11.2, 4.4 Hz, 2H, H-5a, H-5'a), 4.17 - 4.09 (m, 2H, H-5''a, H-3'''), 3.99 - 3.89 (m, 4H, H-5b, H-5'b, H-5''b, H-5'''a), 3.75 (dd,  $J$  = 12.1, 4.8 Hz, 1H, H-5''b) ppm. **<sup>13</sup>C NMR** (100 MHz, CDCl<sub>3</sub>)  $\delta$  166.7, 166.0, 165.8, 165.8, 165.5, 165.4, 165.3, 137.5, 133.8, 133.7, 133.6, 133.5, 133.4, 133.4, 130.0, 130.0, 129.9, 129.4, 129.3, 129.2, 129.1, 128.7, 128.6, 128.5, 128.5, 128.5, 128.4, 127.9, 127.8, 106.0, 105.9 (C-1', C-1''), 105.3 (C-1'''), 105.0 (C-1), 86.3 (C-2'''), 84.5 (C-4'''), 82.3, 82.2 (C-4, C-4'), 82.0 (C-4''), 81.9, 81.8, 81.7 (3  $\times$  C-2), 77.4 (2C, C-3, C-3'), 77.5 (C-3''), 76.8 (C-3'''), 68.7 (CH<sub>2</sub>Ph), 66.2, 66.1 (C-5, C-5'), 65.9 (C-5''), 62.3 (C-5''') ppm. **IR** (neat, cm<sup>-1</sup>): 1721, 1268, 1110, 710. **ESIMS**:  $m/z$  calcd for [C<sub>76</sub>H<sub>68</sub>NaO<sub>24</sub>]<sup>+</sup>: 1387.3993 [M+Na]<sup>+</sup>, found: 1387.3995.

**(171) Phenyl 2,3,5-tri-*O*-benzoyl-1-thio- $\alpha$ -L-arabinofuranoside**<sup>150</sup>

By using the synthetic procedure described for the D-enantiomer,<sup>203</sup> the title compound **171** was obtained in 77% yield as a colorless oil. The analytical data is in accordance with the data for the title compound ( $R_f$  0.62 (3:2 hexane-EtOAc);  $[\alpha]_D^{20} - 52.8$  ( $c$  1.0  $\text{CHCl}_3$ )).<sup>150</sup>

$R_f$  0.22 ( $\text{CH}_2\text{Cl}_2$ ),  $[\alpha]_D^{20} - 49$  ( $c$  2.0  $\text{CHCl}_3$ ).  **$^1\text{H}$  NMR** (300 MHz,  $\text{CDCl}_3$ )  $\delta$  8.19 - 8.09 (m, 2H), 8.08 - 7.96 (m, 4H), 7.68 - 7.27 (m, 14H), 5.84 (s, 1H, H-1), 5.73 (d,  $J = 1.3$  Hz, 1H, H-2), 5.66 (dd,  $J = 4.7, 1.3$  Hz, 1H, H-3), 4.91 - 4.82 (m, 1H, H-4), 4.82 - 4.70 (m, 2H, H-5a+b) ppm.  **$^{13}\text{C}$  NMR** (75 MHz,  $\text{CDCl}_3$ )  $\delta$  166.4, 165.8, 165.6, 133.9, 133.9, 133.7, 133.3, 132.5, 130.3, 130.1, 130.0, 129.9, 129.3, 129.2, 129.1, 128.8, 128.8, 128.6, 128.1, 91.7 (C-1), 82.8 (C-2), 81.4 (C-4), 78.4 (C-3), 63.7 (C-5) ppm. **IR** (neat,  $\text{cm}^{-1}$ ): 3323, 1719, 1262, 1093, 707.

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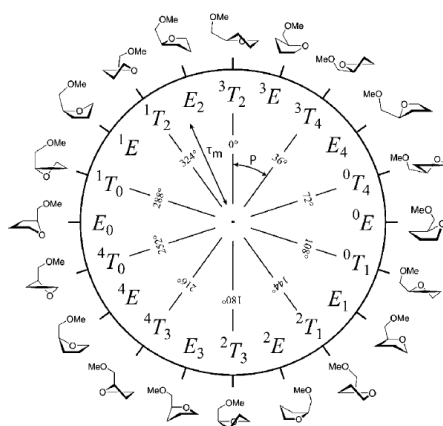
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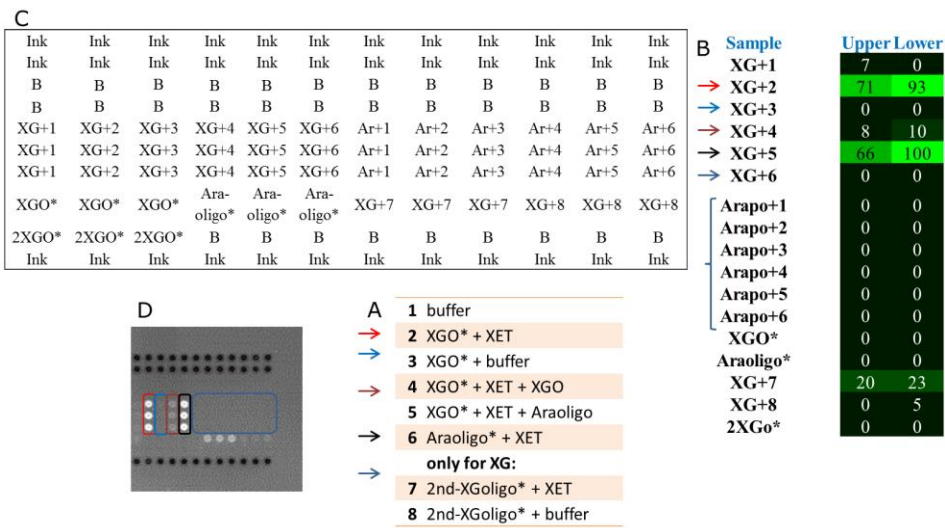
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## Appendix A

Pseudorotational wheel showing the ten envelope and ten twist conformations of furanosides.<sup>208</sup>



Appendix B



Extended version of Figure 28

A) Overview of the incubation conditions used for each sample, with color codes referring to the heat map B. B) Heat map of the scanned array. Upper and lower refer to highest and lowest value in a triplicate set. C) The scanned array. D) The location of each sample on the array.

## Appendix C

**Mathilde Daugaard** and Mads H. Clausen. "Chemical synthesis of (1→5)- $\alpha$ -L-oligoarabinofuranosides." *In preparation*.

Chemical synthesis of oligo (1→5)- $\alpha$ -L-arabinofuranosidesM. Daugaard<sup>a</sup> and M. H. Clausen<sup>\*a</sup>

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Linear (1→5)- $\alpha$ -L-arabinans are commercially available up to the octamer. Branched structures are, however, not accessible. This work describes a strategy for rapid assembly of branched L-arabinans. We demonstrate the utility by synthesizing two branched (1→5)- $\alpha$ -L-octa-arabinofuranosides. The modular approach offers multiple branching possibilities by employing slight modifications.

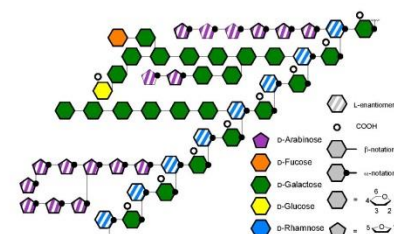


Figure 1 A schematic representation of rhamnogalacturonan-I (RG-I), a part of pectin.

## Introduction

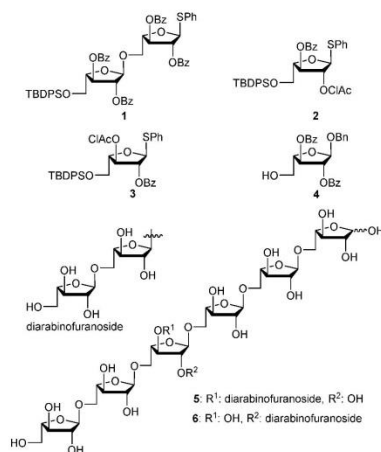
Pectins constitute an important part of the plant cell wall.<sup>1</sup> Investigating the structure of pectin can contribute with more knowledge of properties such as cell wall biosynthesis and protein-carbohydrate interactions. Chemical and/or enzymatic degradation of the cell wall followed by extensive purification can afford some oligosaccharide structures, however in general this only result in minor quantities. Chemical synthesis, on the other hand, is capable of producing structurally diverse and specific oligosaccharides of excellent purity and in larger quantities.

Pectins are built up of different regions, one being rhamnogalacturonan I (RG-I), which holds a backbone of alternating galacturonic acid and rhamnose monosaccharides branched on the rhamnose residues with side chains of linear and branched galactans, arabinans, and arabinogalactans (see Figure 1). The arabinan side chains in the RG-I region are (1→5)-linked  $\alpha$ -L-arabinans branched in the 2- and/or the 3-position.<sup>2,3</sup> So far not many synthesized oligo-L-arabinofuranosides are

reported.<sup>4,5</sup>

Extensive work has, however, been carried out with oligomers of D-arabinofuranosides that make up part of the cell wall of *Mycobacterium tuberculosis*.<sup>6–13</sup>

The synthetic strategy in this work involves four building blocks: a disaccharide donor **1**, two differently protected monosaccharides **2** and **3**, and a reducing end monosaccharide **4** (Figure 2). Employing a disaccharide donor offers a blockwise approach that decreases the number of glycosylations. The orthogonal chloroacetyl protecting group on **2** and **3** leaves a position for introducing branching. The two differently

Figure 2 Building blocks involved in the synthetic strategy of (1→5)- $\alpha$ -L-arabinofuranosides and target molecules **5** and **6**.

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<sup>†</sup> Electronic Supplementary Information (ESI) available: Synthetic procedures and copies of <sup>1</sup>H and <sup>13</sup>C NMR spectra. See DOI: 10.1039/b000000x/

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protected monosaccharides **2** and **3** can be employed different places in the arabinan backbone to obtain core oligosaccharides that can be branched in the 2- or the 3-position with different side chains.

Initially we have used disaccharide donor **1** as side chain in a core hexasaccharide to synthesize two substituted octasaccharides (**5** and **6**) (Figure 2).

## Results and discussion

## Synthesis of monosaccharide building blocks

Reducing end monosaccharide **4**, *N*-phenyl-trifluoroacetimidate donor **10**, and thioglycoside acceptor **9** were all synthesized from TBDPS protected **7** which was obtained in only two steps from L-arabinose (Scheme 1), inspired by Zhang *et al.*'s work on D-arabinose.<sup>14</sup> The first step took advantage of TBDPS's preference for the 5-position and then the monosaccharide was perbenzoylated. Importantly in order to avoid silylating the 3-position, TBDPSCI had to be added slowly to the reaction mixture.

To obtain **4** it was first planned to selectively debenzoylate the anomeric position of **7** to introduce the benzyl group. Experiments with  $\text{BF}_3 \cdot \text{Et}_2\text{O}$ , dimethylamine in water, hydrazine-acetate in dimethylformamide (DMF), and ethane-1,2-diamine, however, did not give any good results. Following investigations showed that it was possible to benzylate **7** directly using  $\text{BF}_3 \cdot \text{Et}_2\text{O}$ , to achieve the benzyl arabinoside in 89% yield. The TBDPS group was removed using tetra-*n*-butylammonium fluoride (TBAF), affording **4** in 90% yield.

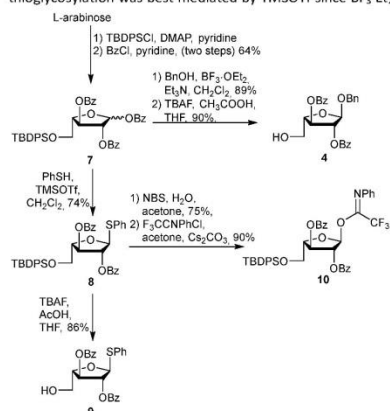
Thioglycoside **8** was obtained from **7** in a similar fashion; thioglycosylation was best mediated by TMSOTf since  $\text{BF}_3 \cdot \text{Et}_2\text{O}$

resulted in poor conversion and many different by-products (Scheme 1).

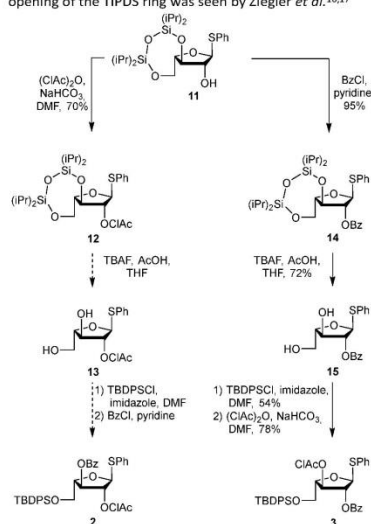
*N*-phenyl-trifluoroacetimidate donor **10** was obtained from thioglycoside **8** by hydrolyzing the anomeric position with *N*-bromosuccinimide (NBS)/water before introducing the acetimidate group. Thioglycoside **8** was treated with TBAF that removed the TBDPS group and afforded acceptor **9** in 86%.

It was envisioned that the two differently protected monosaccharides **2** and **3** could be synthesized from the same protected monosaccharide **11** (Scheme 2).<sup>4</sup> First the 2-position was protected with a chloroacetyl or a benzoyl protecting group, then the TIPDS group was removed and the primary position was selectively protected with a TBDPS group before introducing either a benzoyl or chloroacetyl protecting group on the free 3-position. This strategy gave satisfactory yields for monosaccharide **3** (Scheme 2).

However, removing the TIPDS group from **12** with a chloroacetyl group in the 2-position, only resulted in 45% yield of diol **13**. Diol **18**, where acetic acid has reacted with the chloroacetyl group, was found as a major by-product, see Scheme 3. Gu *et al.* actually reports that TBAF is able to remove the chloroacetyl group completely.<sup>15</sup> It was tested if  $\text{HF} - \text{water}$  could remove the TIPDS group, but this instead resulted in opening of the TIPDS group to afford **16**. A similar opening of the TIPDS ring was seen by Ziegler *et al.*<sup>16,17</sup>



Scheme 1 Synthesis of monosaccharide building blocks.

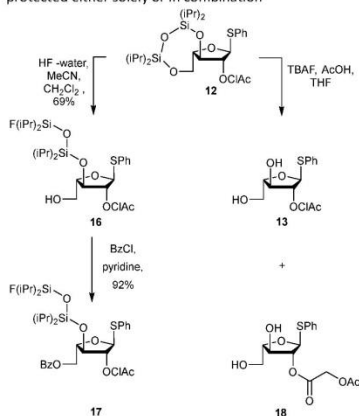


Scheme 2 Synthesis of monosaccharide **3** and the proposed route to monosaccharide **2**.

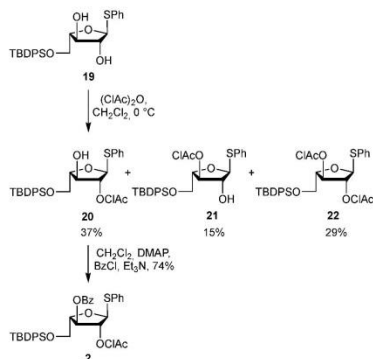


The structure of **16** was verified by nuclear magnetic resonance (NMR) after reacting the free alcohol with benzoyl chloride, which clearly protected the 5-position to obtain **17** (Scheme 3).

Instead monosaccharide **2** was synthesized from the TBDPS protected diol **19** (Scheme 4). It was envisioned that reacting **19** with chloroacetyl anhydride could result in some selectivity for the 2-position either due to steric or electronic factors, or a combination thereof. The reaction showed a slight selectivity for protecting the 2-position, however, the 3-position was also protected either solely or in combination



Scheme 3 By-products from deprotection of the TIPDS group of **12**.



Scheme 4 Synthesis of monosaccharide **2** in two steps from diol **19**.

with the 2-position resulting in **21** and **22**. Since it was possible to separate the two regioisomers **20** and **21** using column chromatography, and since **20** is only one step from monosaccharide **2**, this route was followed despite the low yield. Reacting **20** with benzoyl chloride using 4-dimethylaminopyridine and triethylamine as bases resulted in monosaccharide **2** in 74% yield (Scheme 4). It was found necessary to use milder conditions than standard benzylation conditions using benzoyl chloride in pyridine, which degraded the starting material.

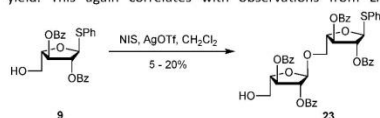
By protecting the 2-position of by-product **21** with a benzoyl group a second route to **3** was found, and the by-product found advantages this way. Bis chloroacetyl protected **21** was used to synthesize a third hexasaccharide backbone, which could be branched in both the 2- and the 3-position.

#### Synthesis of disaccharide donor **1**

To reduce the following number of glycosylations a disaccharide thioglycoside was the first target molecule. Initially it was examined if it was possible to synthesize disaccharide **23** from only one monosaccharide building block **9** (Scheme 5). The glycosylation promoted by a combination of NIS and either silver trifluoromethanesulfonate (AgOTf) or triethylsilyl trifluoromethanesulfonate (TESOTf) yielded the combined donor and acceptor **23** in maximum 20% yield.

The next approach explored the possibility of using thioglycoside **9** as an acceptor in combination with different thioglycoside donors (Scheme 6). The *tert*-butyldiphenylsilyl (TBDPS) protected thioglycoside donor **8** led to the desired disaccharide **1** in 35% yield under optimized conditions. The proportion of TESOTf was varied from 0.1 to 1 equivalents. The major by-product was the trisaccharide thioglycosides **27**, which either resulted from self-coupling of **9** before reacting with the TBDPS protected thioglycoside **8** or the desired product **1** reacting once again with acceptor **9**. Initial experiments with the double TBDPS protected donor **24** showed promising results, which correlates with Liang *et al.*'s findings for the *D*-enantiomer, where *D*-**24** is more reactive than the corresponding 3,5-di-*O*-benzyl protected counterpart.<sup>18</sup> **24** was synthesized from **14**, which was desilylated before the now free 3- and 5-positions were protected with TBDPS.

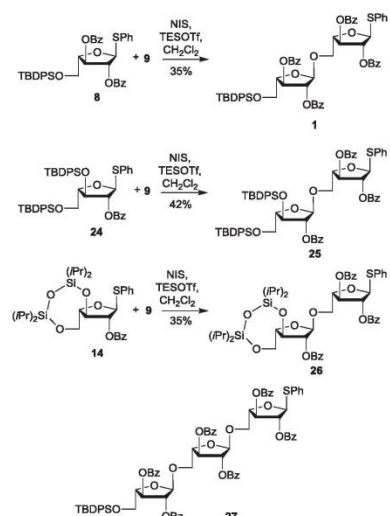
Glycosylation with tetraisopropylidisiloxane-1,3-diyl (TIPDS) protected thioglycoside **14** resulted in disaccharide **26** in 35% yield. This again correlates with observations from Liang



Scheme 5 Glycosylation of **9** with itself to synthesize disaccharide **23**.

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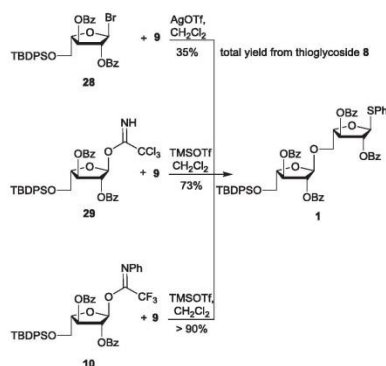


Scheme 6 Coupling between thioglycoside acceptor 9 and different thioglycoside donors.

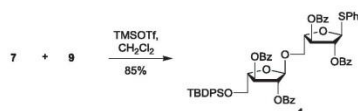
*et al.*'s,<sup>18</sup> who found D-14 to be less reactive than D-24, but more reactive than the thioglycoside protected with the more strained 3,5-di-*O*-*tert*-butylsilylene ring.

To eliminate the possibility of self-coupling of thioglycoside acceptor 9 different glycosylation conditions were examined using three different 5-*O*-TBDPS protected donors. Initial results with the bromide- (28), the trichloroacetimidate- (29), and the *N*-phenyl-trifluoroacetimidate- (10) donors suggested that the *N*-phenyl-trifluoroacetimidate donor 10 was superior yielding disaccharide donor 1 in more than 90% (see Scheme 7). With a good yielding synthetic strategy for the disaccharide donor in hand the monosaccharide building blocks were up scaled.

Thioglycosylation of 7 on a bigger scale resulted in the formation of a by-product, which was identified as disaccharide donor 1. Although the acidic reaction conditions introduced the thiophenyl group, they also slowly removed the TBDPS protecting group, furnishing thioglycoside acceptor 9. This encouraged us to examine the donor properties of 7 carrying a benzoyl group at the anomeric position. Indeed glycosylation between 7 as a donor and 9 as an acceptor was possible yielding disaccharide donor 1 in 85% on a 20 g scale (Scheme 8). Since this strategy involved fewer steps it was desired over the one using the *N*-phenyl-trifluoroacetimidate donor 10.



Scheme 7 Examining different donors to find a good route to disaccharide donor 1.



Scheme 8 Synthesis of disaccharide donor 1 from benzoyl donor 7.

#### Sequential glycosylations and deprotections to give octasaccharides 5 and 6

With all the necessary mono- and disaccharide building blocks in hand we next turned our attention to the oligosaccharide synthesis. First the reducing end trisaccharide 30 was synthesized from disaccharide donor 1 and benzylsaccharide acceptor 4 in 74% yield (Scheme 9). The TBDPS group was removed using TBAF in tetrahydrofuran (THF) furnishing trisaccharide acceptor 31 in 90% yield. Glycosylation between 31 and monosaccharide 3 or 2 resulted in tetrasaccharides 32 or 37, both in 80% yield. The TBDPS group needed to be removed to enable the next glycosylation reaction. As TBAF in THF also reacts with the chloroacetyl group like previously described another fluoride source was needed. HF – pyridine proved to be a suitable choice and resulted in 33 and 38 in 80% and 90%, respectively. Glycosylation with disaccharide donor 1 gave the two core hexasaccharide backbone structures 34 and 40.

To enable branching of the hexasaccharide the chloroacetyl group needed to be selectively deprotected and test reaction were performed on monosaccharide 3. A variety of the most common conditions were investigated. Thiourea unfortunately only resulted in 25% yield (Table 1, entry 1).<sup>19</sup> Lefebvre *et al.*'s conditions (1,4-diazabicyclo[2.2.2]octane (DABCO) in

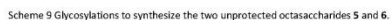
ethanol)<sup>20</sup> showed promising results (Table 1, entry 2) on the monosaccharide. However, the yield dropped when the hexasaccharide was subjected to a variation of the same reaction conditions, in which THF was needed as a co-solvent in order to dissolve the starting material. Instead a dilute solution of NaOMe in THF was tested on monosaccharide **3** (Table 1, entry 3). The chloroacetyl group was removed, but the yield was not better than with DABCO. It was also envisioned that it would be hard to control the unwanted removal of the numerous benzoyl groups on the hexasaccharide. Next the attention was turned to sodium borohydride as a dechloroacetylating reagent (Table 1, entry 4).<sup>21</sup> The conditions showed promising results, even though the solvent was changed from ethanol to a 1:1 mixture of THF-ethanol to get reliable conditions for the hexasaccharide. Finally triethylamine in MeOH/THF was examined and these conditions resulted in 82% of the monosaccharide alcohol and 76% of the hexasaccharide alcohol. The inspiration to try the latter conditions came from obtaining unsatisfying glycosylation results, when the reactions were quenched with triethylamine. After this realization the glycosylation reaction was poured into a mixture of 10% Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub> and a solution of sat. aq. NaHCO<sub>3</sub> upon completion of reaction.

With satisfying conditions for chloroacetyl removal in hand, the final glycosylation to obtain the two octasaccharides could be performed. As the free hydroxyl groups on the last acceptors were more sterically hindered than the ones on the previous acceptors the glycosylations turned out to be more cumbersome. However raising the temperature from around -60 °C to -35 °C and increasing the reaction time made it possible to obtain **36** in 60% yield. **41** was synthesized in 5% yield!

The protecting groups were removed in the following order: 5-O-TBDPS groups, the anomeric benzyl group and last the benzoyl groups, to keep the intermediate compounds as apolar as possible, in order to facilitate handling and purification by chromatography on Silica. Without the complication from the chloroacetyl group, the TBDPS groups were removed using TBAF in THF. The anomeric benzyl group turned out to be a bit more difficult to remove. Palladium on carbon in H<sub>2</sub> atmosphere in different solvents and under different temperatures either led to no conversion or decomposition of the starting material at higher temperatures. Instead palladium hydroxide on carbon in ethanol and ethyl acetate was tried and gave the triol as the only product. Finally the benzoyl groups were removed using the Zemplén conditions and the target molecules **5** and **6** were synthesized in xx% and xy% over three steps.

Table 1 Conditions to remove the chloroacetyl group from monosaccharide **3** and **34**.

Entry	Conditions	Mono-saccharide <b>3</b>	Hexa-saccharide <b>34</b>
1	THF, thiourea, Bu <sub>4</sub> Ni, NaHCO <sub>3</sub>	25%	
2	DABCO, EtOH/THF 2:1	75%	68%
3	0.02 M NaOMe, THF	71%	
4	NaBH <sub>4</sub> , EtOH/THF 1:1	80%	
5	Et <sub>3</sub> N, MeOH/THF 1:1	82%	76%



### Conclusions

In summary, we report here a concise synthetic strategy for two (1→5)-linked  $\alpha$ -l-octaarabinofuranosides related to the plant polysaccharide pectin. Two different core hexasaccharide backbones were branched on the fourth monosaccharide on either the 2- or the 3-position with a diarabinofuranoside. Furthermore the synthetic route allows one to branch the hexasaccharide backbone with a variety of different building blocks.

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